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Hypophysectomy in Mice with Special Reference to Mammary Cancer

A Report on the Outcome of 351 Operations

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These investigations were undertaken to ascertain (a) the fate of mice with either a high or low predisposition to cancer of the mammary gland following extirpation of the hypophysis, and (b) the fate of hypophysectomized mice of a high cancer predisposition bearing transplanted hypophyses from mice of a low cancer predisposition and vice versa. Although the average time of survival was too short to allow decisive conclusions so far as the occurrence of cancer was concerned, we believe that the data obtained are of sufficient importance to record here because they provide valuable information for further experiments. The work was carried out during the years 1937-1940.

METHOD

The technic of hypophysectomy has been described elsewhere (7), and was used in a later work (5) concerning cancer in hypophysectomized mice. The topography of the hypophysis in the mouse is shown clearly in Figs. 1 and 2.

Owing to the great operative mortality in dba mice we operated upon some of them in two stages (see Table 1, Group V), with better results. In these cases actual hypophysectomy was carried out one or two days after the bone panel had been removed from the base of the skull.

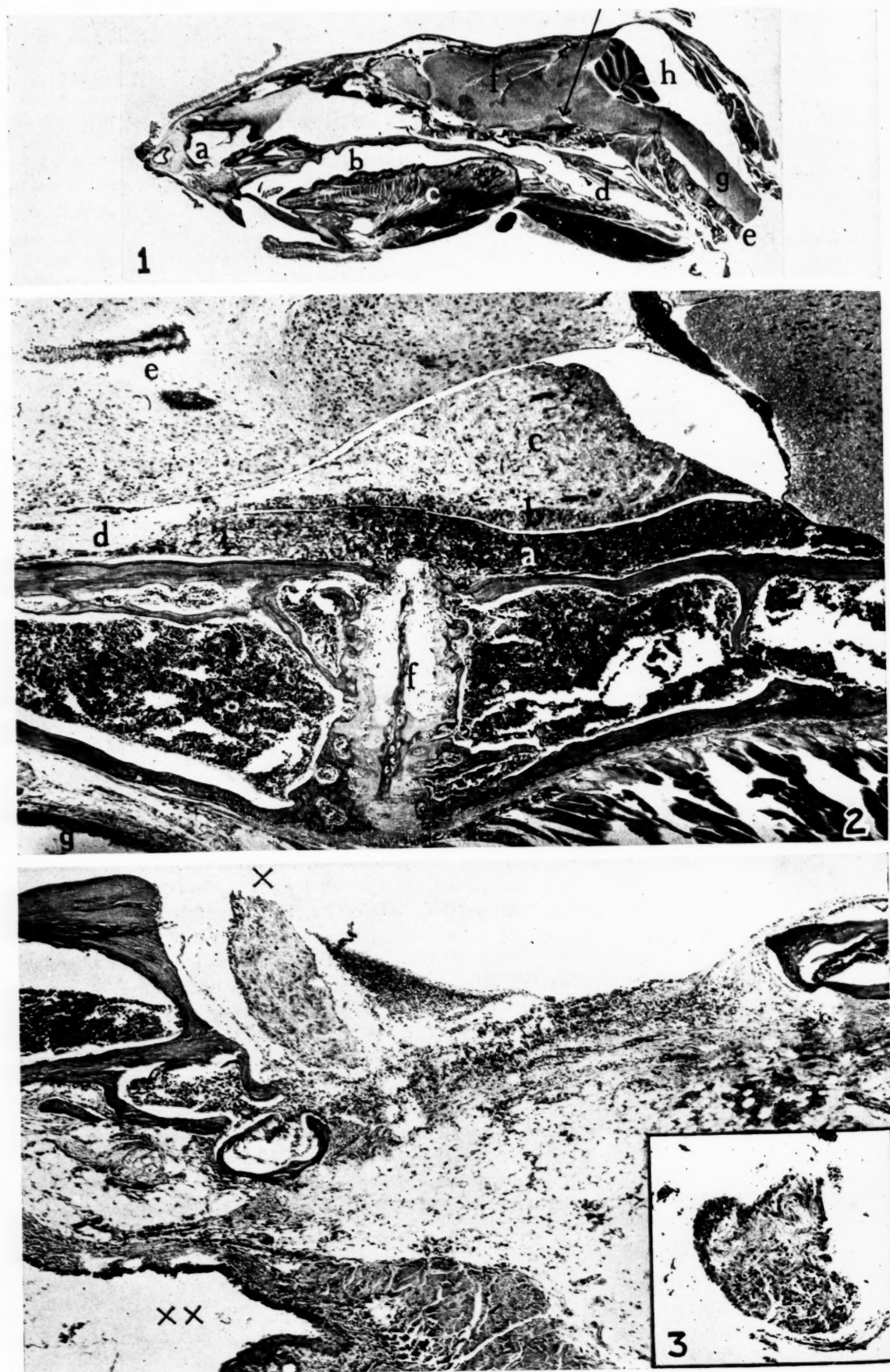
The grafting of the hypophysis was carried out in the following manner. The vault of the cranium with part of the brain was removed with scissors from the donor, and the remainder of the brain removed from the base of the skull with a blunt hook.

The hypophysis was extracted with a pin and placed in saline. A midline incision was made in the abdominal skin of the anesthetized receptor and the skin loosened from the underlying muscles. The hypophysis was then deposited in this pouch, which was finally closed by two sutures.

Operative results.—In a large number of cases post-mortem examination of the skull was carried out, the base being minutely explored with a binocular microscope to identify any remaining hypophyseal fragments. Whenever a small, white, suspicious mass was found, or the stump of the stalk seemed too thick, a microscopic examination was made of serial sections of this mass and of the adjoining part of the base of the skull. If only a few cells belonging to the pars tuberalis or the pars intermedia were found clinging to the stalk of the hypophysis we considered the operation successful (Figs. 3a and 3b). If, on the other hand, anterior lobe tissue was found we regarded it as unsuccessful (Figs. 4, 5, 6).

Strains of animals.—Experiments were carried out on mice of the "dilute brown, Murray-Little" (dba) and "C57 black Little" (B) strains and their F₁ hybrids. The hybrids are designated as F₁dB when their mothers were from strain dba, and as F₁Bd when their mothers were from strain C57 black.

The incidence of mammary cancer is very high in dba and F₁dB females, and very low in B and F₁Bd females (4). In addition, experiments were performed with F₁ hybrids, the mothers of which belonged to strain "O₂₀Leeuwenhoekhuis" (2) and the fathers to strain dba. These, designated as F₁Od, have a low incidence.



FIGS. 1-3

RESULTS

A total of 351 female mice were hypophysectomized. The results are summarized in Table I, which includes mice hypophysectomized only and others hypophysectomized after having received implants of hypophyses from other mice. The outcome presents a good idea of what an experienced operator can achieve with this technic.

the number in column 8 with respect to the number in column 3 diminished by the number in column 7).

Postmortem examination of the skull was not carried out in every instance and the percentages are probably a little high, because some incomplete hypophysectomies may have escaped our notice. Since the numbers of mice in the various groups are far too small for statistical analysis these percentages are given only to make the material easily comparable.

TABLE I: SUMMARY OF HYPOPHYSECTOMIZED FEMALE MICE

1 Group	2 Genetic constitution of hypophysectomized mice and donors of hypophyses		3 No. of mice	4 No. dead first 3 days	5 No. dead 4 to 20 days	6 No. surviving more than 20 days	7 No. incompletely hypophysec- tomized	8 No. completely hypophysec- tomized	9 % Surviving hypophy- sectomy more than 20 days
	Hypophysec- tomized	Donors							
I	F ₁ dB	30	1	0	29	0	29	97
II	F ₁ Bd	21	2	0	19	1	18	90
III	B	81	40	4	37	2	35	44
IV	dba	67	40	5	22	0	22	33
V*	dba	20	0	5	15	2	13	72
VI	F ₁ Od	8	1	0	7	0	7	87
Total	227	84	14	129	5	124	55
VII	F ₁ dB	♀ F ₁ Bd	32	0	0	32	4	28	100
VIII	F ₁ dB	♂ F ₁ dB	2	0	0	2	0	2	100
IX	F ₁ Bd	♀ F ₁ dB	38	6	0	32	1	31	84
X	F ₁ Bd	♂ F ₁ Bd	13	0	0	13	0	13	100
XI	F ₁ Bd	♀ dba	6	0	0	6	0	6	100
XII	F ₁ Od	♀ F ₁ Od	6	0	0	6	0	6	100
XIII	F ₁ Od	♂ F ₁ Od	27	4	5	18	2	16	64
Total	124	10	5	109	7	102	87
Total	351	94	19	238	12	226	67

* Group V, operation in 2 stages.

In some of the groups the immediate operative mortality was very high. In the vast majority of cases death occurred on the day of the operation, or in the 3 days following (column 4). From the fourth to the 20th day inclusive the operative mortality was low (column 5) and during the following 2 months very low: 10 out of 238 mice. Of 351 females, 238 survived hypophysectomy longer than 20 days (column 6); in 12 of these (column 7) it was found that the operation had not been complete. In the remaining 226 (column 8) we feel justified in speaking of a successful operation. The percentages of successful operations are given in column 9 (calculated from

Tables II and III include only those mice that survived hypophysectomy longer than 20 days, and in which we thought ourselves justified in considering the operation a success. Table II includes the animals that were hypophysectomized only; Table III the animals in which, before this operation, the hypophysis of another mouse had been implanted subcutaneously. The average age at which hypophysectomy was performed (with the minimum and the maximum age in brackets) is given in column 4. The average duration of life of these mice is found in column 5. Column 6 gives the average duration of life after hypophysectomy. In column 12 of Table III the age

DESCRIPTION OF FIGURES 1 TO 3

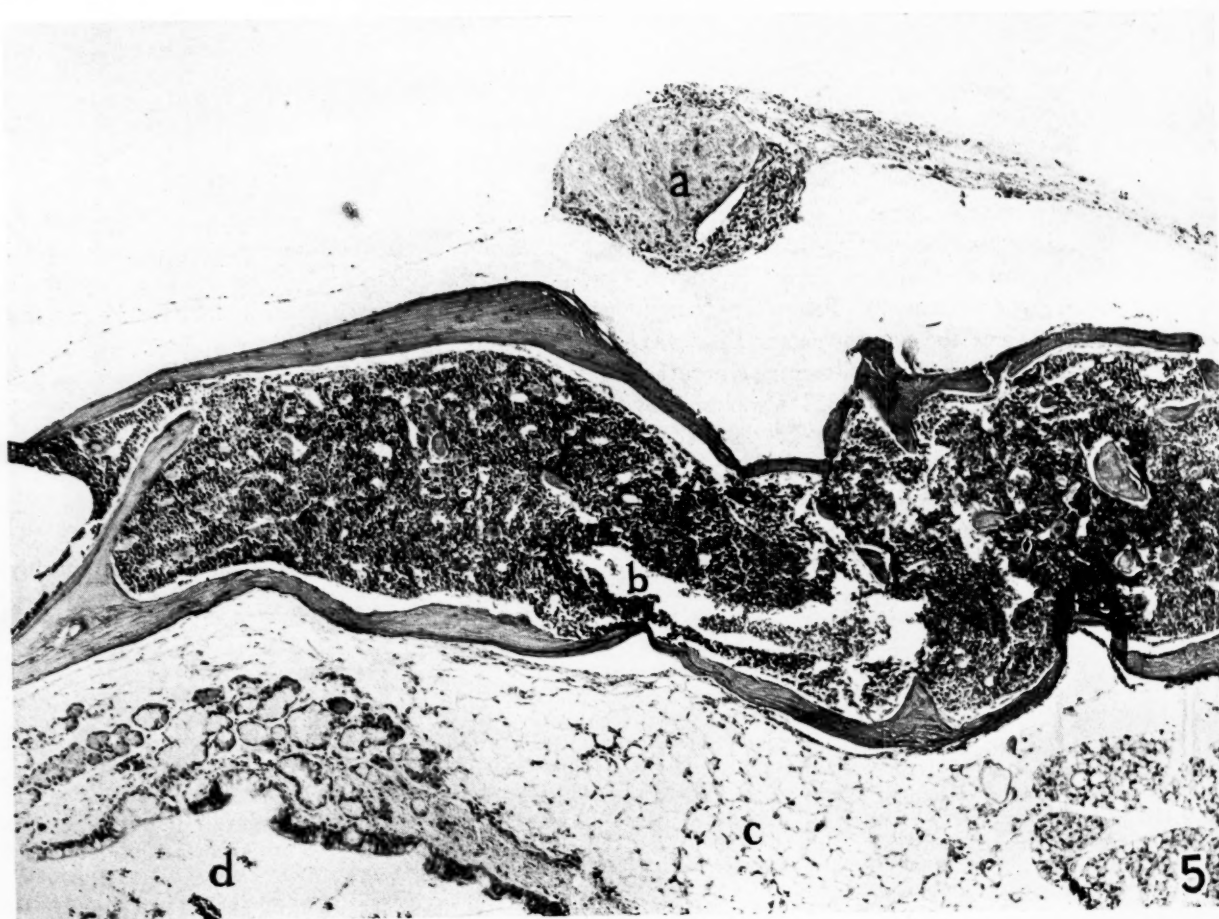
FIG. 1.—Mouse 98692. Median sagittal section through head of dba mouse. a = Nose. b = Mouth. c = Tongue. d = Trachea. e = Spine. f = Brain. g = Cord. h = Cerebellum. Hypophysis indicated by arrow. In dba mice the hypophysis is situated slightly more anteriorly than in other strains. Mag. $\times 106$.

FIG. 2.—Mouse 98692. Detail of Fig. 1, magnification of hypophyseal area. a = Pars anterior. b = Pars intermedia. c = Pars posterior. d = Pituitary stalk. e = Third ventricle.

f = Synchronosis spheno-occipitalis. g = Pharynx. Mag. $\times 106$.

FIG. 3.—Mouse 103. Median sagittal section of base of skull cut serially. X = Hypophyseal stalk (tilted in gap made at operation in base of skull. XX = Pharynx. The operation is considered successful. Mag. $\times 106$.

Insertion: Mouse 48452. Hypophyseal stalk to which a few cells of the middle lobe are still clinging. The operation is considered successful. Mag. $\times 106$.



FIGS. 4-5

is given of those mice that received implants of hypophysis, and column 13 the age of the donors of the hypophyses.

The animals mentioned in these two tables all showed the characteristics of hypophysectomized mice, as described by one of us on a previous occasion (7);

the skull was carried out 93 times (column 10). According to the criteria previously mentioned, hypophysectomy had been invariably successful.

The ovaries were examined microscopically in 32 mice (column 11). No definite corpus luteum and no definite luteinization of the interstitial ovarian

TABLE II: SUMMARY OF SUCCESSFULLY HYPOPHYSECTOMIZED FEMALE MICE WITHOUT IMPLANTS

1	2	3	4	5	6	7	8	9	10	11
Group	Genetic constitution	No. of mice	Age at hypophysectomy, days	Age at death, days	Survival after hypophysectomy, days	Weight at hypophysectomy, gm.	Weight 4 to 10 months after hypophysectomy	No. in which vaginal smears were made	No. in which cranial examination was made	No. in which ovaries were examined
I	F ₁ dB	29	46 (31-77)	376 (240-461)	330	18 (10-24)	16 (10-27)	2	0	0
II	F ₁ Bd	18	55 (33-67)	403 (172-479)	348	17 (15-22)	15 (11-19)	3	1	1
III	B	35	57 (25-89)	220 (79-388)	163	17 (10-22)	14 (9-19)	0	2	0
IVa	dba	19	76 (35-110)	256 (98-324)	180	20 (14-25)	12 (10-15)	0	2	2
IVb	dba	3	364	458	94	24	15	0	0	0
V*	dba	13	120 (89-162)	350 (161-525)	230	23 (20-27)	14 (12-16)	0	2	2
VI	F ₁ Od	7	±28-±42	>273	>231	16 (15-17)	?	3	7	0
Total	124						8	14	5

* Group V, operation in 2 stages.

TABLE III: SUMMARY OF SUCCESSFULLY HYPOPHYSECTOMIZED FEMALE MICE BEARING IMPLANTS OF HYPOPHYSES

1	2		3	4	5	6	7	8	9	10	11	12	13	14	15
Group	Genetic constitution		No. of mice	Age at hypophysectomy, days	Age at death, days	Survival after operation, days	Weight at time of hypophysectomy, gm.	Weight 4 to 10 months after hypophysectomy, gm.	No. in which vaginal smears were made	No. in which cranial examination was made	No. in which ovaries were examined	Age at implantation, days	Age of implant, days	No. in which implant was sought	No. in which implant was found
	Hypophysectomized	Donors													
VII	F ₁ dB	♀ F ₁ Bd	28	72 (40-118)	501 (210-647)	429	20 (16-26)	24 (16-30)	26	22	9	51 (29-87)	111 (85-154)	22	14
VIII	F ₁ dB	♂ F ₁ dB	2	40 (40-41)	418 (397-438)	378	16 (16-17)	21 (17-25)	2	2	2	29 (29-30)	29 (29-30)	2	0
IX	F ₁ Bd	♀ F ₁ Bd	31	72 (50-109)	576 (487-646)	504	20 (15-27)	23 (16-32)	31	24	3	48 (31-93)	137 (66-359)	23	17
X	F ₁ Bd	♂ F ₁ Bd	13	60 (46-83)	505 (327-618)	445	18 (15-21)	21 (14-27)	13	8	8	36 (26-40)	36 (26-40)	9	7
XI	F ₁ Bd	♀ dba	6	113 (110-119)	522 (471-573)	409	21 (19-22)	20 (18-25)	6	5	5	57 (55-63)	46 (41-66)	5	3
XII	F ₁ Od	♀ F ₁ Od	6	±42-±56	>271	>215	15 (13-17)	18 (16-22)	0	6	0	±28-±42	±42-±56	6	4
XIII	F ₁ Od	♂ F ₁ Od	16	±42-±56 14 (10-17)	18 (14-23)	18 (14-23)	0	12	0	±28-±42	±42-±56	16	8
Total			102						78	79	27			83	53

this appeared most clearly in those operated upon when young. In general there was a distinct difference between mice with and without implants, in that the former were more active.

In a number of mice from 6 to 12 months of age vaginal smears were made daily (column 9) for a period of 3 to 5 weeks; in none was any trace of estrus found. A careful postmortem examination of

parenchyma was ever found. We shall refer to this point in the Discussion.

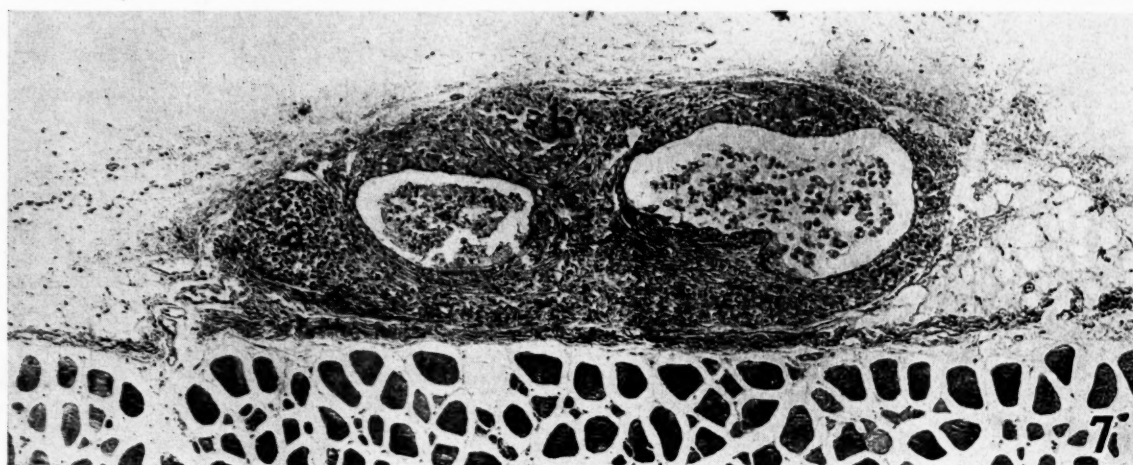
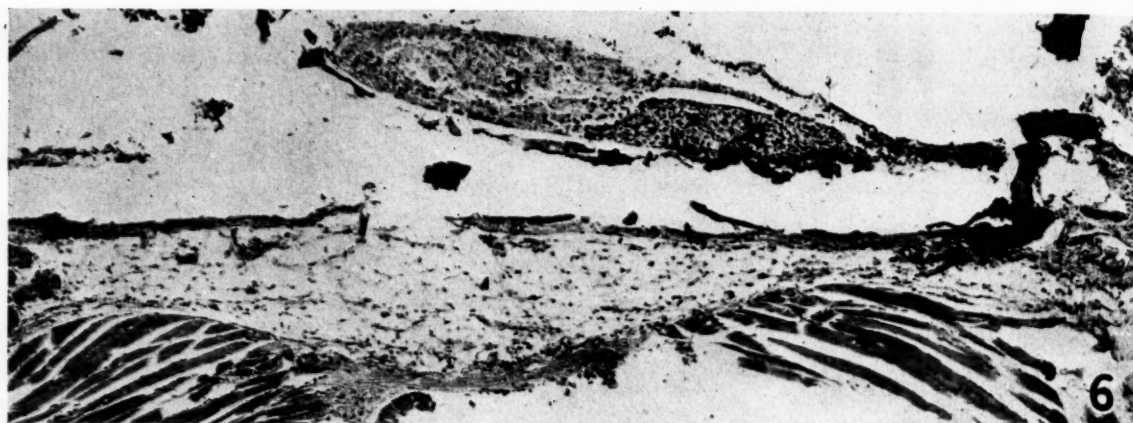
The body weight on the day of operation (column 7) varied greatly, depending mainly on the age when hypophysectomy took place. The body weight 4 to 10 months after hypophysectomy (column 8) had usually decreased in those mice that had been hypophysectomized only, whereas in those in which

DESCRIPTION OF FIGURES 4 AND 5

FIG. 4.—Mouse 47734. Sagittal section of base of skull cut serially. Unsuccessful operation. Reason for failure: hook for hypophysectomy tore through bone of base of skull posterior to sphenoidosis sphenoid-occipitalis (XX) instead of splitting the latter open. Structures underneath bone are peripharyngeal cellular tissue. Hypophyseal fragment (X) is composed of histologically normal anterior lobe parenchyma in which eosinophils predominate. Mag. $\times 106$.

FIG. 5.—Mouse 48142. Sagittal section of base of skull cut

serially. a = Hypophyseal remnant. b = Base of skull. c = Peripharyngeal connective tissue. d = Pharynx. Pituitary remnant consists of stalk to which clings a fragment of anterior lobe, partly separated from the latter by hypophyseal cleft. Anterior lobe parenchyma shows signs of degeneration characterized by disappearance of cell granules and condensation of chromatin. Chromophobes predominate. Eosinophils present. The operation is considered unsuccessful. Mag. $\times 106$.



FIGS. 6-8

a hypophysis had been implanted it had increased slightly. This change will be mentioned in the Discussion.

The number of times we looked for the implant and the number of times we found it will be found in columns 14 and 15 of Table III. The significance of these figures, as well as the histologic picture presented by the implants, will also be discussed later. None of these mice developed cancer of the mammary gland.

A description will now be given of the 12 mice listed in column 7 of Table I, in which the operation had not been complete. A detailed description is given of the hypophyseal remnants found at the base of the skull. As a general preliminary remark we wish to emphasize the fact that every time a fragment of pituitary tissue was found in the cranial cavity it showed signs of degeneration of the same nature as those we shall describe in the implants, with this difference; that in the latter these signs were much more definite.

Group II.—Only 1 mouse, 402 days old, was killed. Mouse 47734, hypophysectomized when 49 days old, was killed healthy when 485 days old. It had increased 4 gm. in weight in 6 months. Estrus had occurred a number of times. Postmortem examination revealed the presence of a fragment of the anterior lobe containing chromophobes, basophils, and numerous eosinophils (Fig. 4). Ovary: Corpora lutea present. No tumor.

Group III.—None of these mice were killed. Mouse 92269, hypophysectomized when 49 days old, reached the age of 503 days. Its weight had increased 7 gm. in 6 months. Estrus had occurred a number of times. Autopsy: At the base of the skull the stalk of the hypophysis was found, with fragments of all 3 lobes attached to it. In the fragment of the anterior lobe chromophobes, basophils, and a few eosinophils were found. Ovary: stroma packed with lutein cells. No tumor.

Mouse 91949, hypophysectomized when 85 days old, died when 633 days old. Weight increase, 7 gm. No estrus detected (3 weeks' examination). Autopsy:

At the base of the skull a fragment of the anterior lobe of the hypophysis was found containing chromophobes, basophils, and a great many eosinophils. Ovary: Stroma closely packed with lutein cells. No tumor.

Group V.—Mouse 96935, hypophysectomized in 2 stages at the age of 162 days, was killed when 457 days old. It had cancer of a mammary gland. Loss of weight, 2 gm. Autopsy: At the base of the skull lay a fragment of the anterior lobe of the hypophysis containing chromophobes, basophils, and numerous eosinophils. Ovary: Numerous corpora lutea were present.

Mouse 96996, hypophysectomized in 2 stages when 118 days old, died when 699 days old (the maximum age reached in this group after successful operation was 525 days). Weight increase, 4 gm. Owing to decomposition, autopsy of the skull was impossible. Ovary: Stroma closely packed with lutein cells. No tumor.

Group VII.—Mouse 48137, which had received the implant when 31 days old and was hypophysectomized when 42 days old, reached the age of 412 days. Weight increase, 14 gm. No estrus was detected (3 weeks' examination). Autopsy: At the base of the skull a fragment of the anterior lobe of the hypophysis was found containing chromophobes and basophils, but no eosinophils. Ovary: Absence of corpora lutea; absence of interstitial luteinization. Implant: Atrophic. No tumor.

Mouse 48142, which had received the implant when 29 days old and was hypophysectomized when 42 days old, died when 410 days of age. Weight increase, 7 gm. Definite estrous cycles. Autopsy: At the base of the skull a fragment of the anterior lobe of the hypophysis was found containing chromophobes, basophils, and many degenerating eosinophils. (Fig. 5). Ovary: Intense interstitial luteinization. Implant: Atrophic. No tumor.

Mouse 48143, which had received the implant when 29 days old and was hypophysectomized when 42 days old, reached the age of 410 days. Weight increase, 5 gm. Definite estrous cycles. Autopsy: At

DESCRIPTION OF FIGURES 6 TO 8

FIG. 6.—Mouse 48048. Sagittal section of base of skull cut serially, in a mouse in which hypophysectomy was not complete. a = Stump of hypophyseal stalk. b = Remnant of intensely vascularized pars anterior. A higher magnification shows it to be constituted of basophils. The latter, judging by size of their idiosomes, present considerable secretory activity. Hypophyseal cleft is clearly visible. The structures underneath the fragment are peripharyngeal connective tissue and muscle. The operation is considered unsuccessful. Mag. $\times 106$.

FIG. 7.—Mouse 48420. This implant, on surface of muscle of abdominal wall, contains 2 cysts. At the pointed end (a) nodule

of anterior lobe can be detected, which consists mainly of chromophobes; it contains only a few eosinophils, poor in granules (cytological characters not recognizable at this magnification). Between the 2 cysts, nodule belonging to pars intermedia can be identified (b). Mag. $\times 106$.

FIG. 8.—Mouse 96. Sagittal section of base of skull cut serially. Division of hypophyseal stalk. Considerably enlarged hypophysis rests in gap made at operation in base of skull. Anterior lobe has retained its normal structure. Enlargement due to hypertrophy of pars intermedia, cells of which present signs of intense secretory activity. Mag. $\times 106$.

the base of the skull a fragment of the hypophysis was found containing parenchyma belonging to all 3 lobes. Chromophobes and basophils, but no eosinophils were present. Ovary: Intense interstitial luteinization. No implant recovered. No tumor.

Mouse 48048 had received the implant when 71 days of age, was hypophysectomized when 102 days of age, and killed when 373 days old. It had a cancer of a mammary gland. No estrous cycles occurred (2 weeks' examination). Autopsy: At the base of the skull the stump of the pituitary stalk was found, with a fragment of the anterior lobe attached to it. (Fig. 6.) Numerous basophils but no eosinophils were present in the latter. The basophils contained a large idiosome, suggestive of considerable secretory activity. Ovary: Absence of any luteinization worth mentioning. The implant was not sought.

Group IX.—Mouse 48185 had received the implant when 39 days old, was hypophysectomized when 68 days old, and killed healthy at the age of 387 days. Loss of weight, 2 gm. Definite estrous cycles occurred. Autopsy: At the base of the skull a fragment of the hypophysis was found containing tissue of all 3 lobes. In the anterior lobe chromophobes and basophils, no eosinophils. Ovary: Intense interstitial luteinization. Implant: Atrophic. No tumor.

Group XIII.—Mouse 61. Hypophysectomy at the age of 4 to 6 weeks, 1 day after implantation. Killed 308 days after hypophysectomy. Autopsy: At the base of the skull the stalk of the hypophysis was found, with a small fragment of anterior lobe attached to it containing basophils and eosinophils. Implant: Atrophic. No tumor.

Mouse 64. Hypophysectomy at the age of approximately 6 weeks, 23 days after implantation. Killed 283 days after operation. Autopsy: No remnant of hypophysis was detected at the base of the skull. Implant not recovered. No tumor. Although we did not find a remnant of the hypophysis, we consider the operation unsuccessful since this mouse, in 10 months, increased from 16 to 35 gm. in weight. We have never observed a similar excessive gain after complete hypophysectomy; in the other mice of this group, the increase in weight reached 8 gm. at the most. Consequently we deem it highly probable that during the autopsy a fragment of the hypophysis was overlooked.

DISCUSSION

Five groups (I, IV, V, VII, VIII) were composed of mice in which a high predisposition to cancer of the mammary gland normally exists. The animals of Groups I, IV, and V were hypophysectomized only. Twenty-four mice of Groups IV and V were given injections of growth hormone for a short period

of time. This resulted in a slight increase in weight, which was lost as soon as the injections were stopped. It seems highly improbable that the injection of these animals with growth hormone should have influenced the result of the experiment. Those of the 2 other groups received implants of hypophyses from females with a low predisposition to cancer (Group VII), or from males of a type in which most of the females develop cancer (Group VIII).

As shown in Table IV, if these had been normal mice the collective tumor probability (3) would have amounted to 16.84; *i.e.*, given an equal number of mice of these types of the same age mammary cancer would have developed in about 17. Actually none developed cancer.

The result in Groups I, IV, and V indicate that the removal of the hypophysis brings about a great decrease in the predisposition to mammary cancer. From the result in Groups VII it is seen that this

TABLE IV: HYPOPHYSECTOMY AND SUBCUTANEOUS IMPLANTATION OF A HYPOPHYSIS IN REGARD TO PREDISPOSITION TO MAMMARY CANCER

1	2		3	4†	5
	Genetic constitution			Expected number of cancers in controls	No. of mice developing cancer
Group	Hypophysectomized	Donor	No. of mice		
I	F ₁ dB	29	2.48	0
IV	dba	22	1.57	0
V*	dba	13	2.34	0
VII	F ₁ dB	♀ F ₁ Bd	28	10.20	0
VIII	F ₁ dB	♂ F ₁ dB	2	0.25	0
Total	94	16.84	0

* Group V, operation in 2 stages.

† See Korteweg (3).

predisposition does not become greater under the influence of a hypophysis implanted subcutaneously from a type in which the predisposition to cancer is low.

We have already drawn attention to the fact that in cases in which we considered the hypophysectomy successful we had not always succeeded in removing *all* the hypophyseal parenchyma. Our experiments prove that mice with only a few cells of the pars tuberalis or the pars intermedia clinging to the stalk behave as animals completely deprived of the hypophysis. None ever showed estrus. The growth of all lagged far behind that of normal mice of the same type, and all showed the habitus characteristic of hypophysectomized animals.

Five groups (II, III, IX, X, XI) include mice with a very low predisposition to mammary cancer. The animals of Groups II and III had been hypophysectomized only. Those of the other groups had also been implanted with the hypophysis of a female (Groups IX and XI) belonging to a type with a high predisposition to cancer, or of a male (Group X)

belonging to a type with a low predisposition. In none of these 103 mice, many of which had advanced far into the cancer age, did a mammary cancer develop.

From the result in Groups II and III it follows that removal of the hypophysis in mice of a type with a very low predisposition to mammary cancer causes no increase in their predisposition worth mentioning. The result in Groups IX and XI shows that when the pituitary of a mouse with a high predisposition has been implanted in these hypophysectomized animals; the predisposition still remains very low.

The conclusion to be drawn is, that the predisposition to mammary cancer is low in hypophysectomized mice that belong to a type with a high predisposition to this kind of tumor under normal circumstances. Subcutaneous implantation of hypophyses of other mice does not alter the low predisposition following hypophysectomy.

3. What influence does a hypophysis implanted subcutaneously exercise on the hypophysectomized mouse?

In answer to the first question, we include the results obtained with Groups I to V, and VII to XI. (We do not know with sufficient certainty at what ages the mice of Groups VI, XII, and XIII were hypophysectomized.) In our calculations we have included only those mice in which incomplete hypophysectomy seemed improbable, though admitting that among this material there may have been a few mice in which a fragment of the gland was left behind. Neither have we included the 3 mice of Group IV on which hypophysectomy was performed on the 364th day of life.

Our calculations comprise 194 mice, belonging to 3 genetic types and are summarized in Table V. Column I gives the age at which hypophysectomy was performed. Columns 2, 3, 4, and 5 give for each genetic type the number of mice and the age

TABLE V: DURATION OF LIFE, HYPOPHYSECTOMIZED MICE WITH OR WITHOUT IMPLANTS

1 Age at hypophysectomy, days	2a 2b		3a 3b		4a 4b		5a 5b	
	Group III no implant, B		Groups IV-V no implant dba		Groups I-II no implant, F ₁ dB and F ₁ Bd		Groups VII-XI implant, F ₁ dB and F ₁ Bd	
	No. of mice	Mean age at death, days	No. of mice	Mean age at death, days	No. of mice	Mean age at death, days	No. of mice	Mean age at death, days
20-29	5	168
30-39	4	187	2	148	12	348
40-49	4	228	9	364	13	473
50-59	8	208	2	261	18	428	14	564
60-79	6	239	7	257	8	402	24	550
80->	8	250	21	312	29	531
Total	35	...	32	...	47	...	80	...

Whether the pituitary hormones influence this predisposition directly or whether they work circuitously only, as, for instance, by means of the ovary, cannot be deduced from our experiments. In a former experiment we found, however, that of 71 castrated dba females (at ages varying from 53 to 145 days) 29 developed mammary cancer, whereas in Groups IV and V of the experiment described herein not one of the 35 dba females that had been hypophysectomized at ages ranging from 35 to 162 days (and 3 at the age of 1 year) did so. However, the duration of life of dba mice is so much shortened by hypophysectomy that hardly a mouse of this strain reaches the age at which the first cancers arise in castrated dba females.

The problems raised at the outset have been practically answered. Our material also enables us to answer the following questions:

1. What influence has hypophysectomy, performed at varying ages, on the duration of life of mice?
2. What happens to a hypophysis implanted subcutaneously?

at which the operation was performed, as well as the average age attained. Since some mice were killed when healthy a correction has been made. For these animals, which were among those living longest, the age actually reached has arbitrarily been raised by 20 days.

In general normal F₁dB and F₁Bd mice, provided the former do not develop mammary cancer, attain an age of over 2 years. This is also the case with normal B mice, and ages of 3 years are no exception in them. Of dba females the vast majority develop mammary cancer, and for this reason few live to be 2 years old; only a few die under 1 year of age. From Table V it may be concluded that the chances of life of these mice are very much shortened by hypophysectomy, and, it is clear also that the older they are when hypophysectomy is performed the longer they live.

The hybrid F₁ mice appear to withstand hypophysectomy much better than dba or B mice; this can be attributed to the greater vitality of the F₁ animals, which is apparent in other respects also.

In answer to the second question, which concerns the fate of the implanted hypophysis, we refer to the results of Groups VII to XIII, shown in columns 14 and 15 of Table III. The figures stating how often implantation was successful are minimum figures because in a very careful examination the chance of overlooking the implant, which is very small, and has the aspect of a tiny flattened spindle embedded in the abdominal muscle, is not to be underestimated.

Excluding those mice in which we did not look for the implant because of decomposition or other reasons, 83 animals remain. These belong to 2 genetically different types, in which the implant was found 53 times (*i.e.*, 64 per cent of the cases) and examined microscopically. The percentages in which the implant was found in the various groups do not differ greatly, neither is there any great difference according to whether the implant came from a male or a female donor.

TABLE VI: RESULTS OF SUBCUTANEOUS IMPLANTATION OF HYPOPHYSES IN HYPOPHYSECTOMIZED MICE

1	2a	2b	2c	3a	3b	3c
	Groups VII-XI F ₁ dB and F ₁ Bd			Groups XII-XIII F ₁ Od		
Age at death, days	No. implants sought	No. implants found	% Positive	No. implants sought	No. implants found	% Positive
-400	5	2	40	22	12	55
401-500	13	6	46
501-600	29	22	76
601->	14	11	79
Total	61	41	68	22	12	55

The percentage of successful subcutaneous implantations of hypophyses was approximately equal to that obtained by A. E. Nordholt, in this laboratory, who succeeded in transplanting an ovary or an epididymis from one mouse to another (6).

The hypophyses employed came from mice 26 to 154 days of age and, in addition, from 4 that were about 360 days old. The 4 implants last mentioned were found again, 455 to 562 days after implantation into mice from 505 to 612 days old. This proves that old hypophyses also grow satisfactorily. Furthermore, we found no correlation between the interval of time elapsing between hypophysectomy and implantation and the chance that the implant would take.

Table VI shows to what extent a correlation exists between the age of the new host and the chance of finding the implant. Because of the comparatively small numbers of mice, we totalled the groups of equal genetic type. The table reveals that a successful hypophyseal graft does not, as a rule, disappear later in life.

Histologically all implants showed signs of regression. The pars nervosa had disappeared and the an-

terior lobe had undergone considerable degeneration (Fig. 7). The nuclei were pyknotic and the granulation had pretty well disappeared. The number of eosinophils had greatly diminished. Chromophobes predominated. The cells of the pars intermedia remained recognizable longest. The cleft of the hypophysis in many cases showed cystic dilatation, and the general picture resembled that described by Wolfe, Kirtz, and Loeb (8).

What influence has an hypophysis implanted subcutaneously on the hypophysectomized mouse?

To decide this we compared the results obtained with the Groups VII to XI with those obtained with the Groups I and II. We have already pointed out that mice bearing the implants were livelier on the whole than those of the same type with none.

To what extent the implant influences the longevity of the host may be gathered from Table V, columns 4 and 5. It appears that the average age

TABLE VII: CHANGES IN WEIGHT IN HYPOPHYSECTOMIZED MICE WITH OR WITHOUT IMPLANTS 4 TO 6 MONTHS AFTER OPERATION

1	2a	2b	3a	3b
	Groups I-II F ₁ dB and F ₁ Bd		Groups VII-XI F ₁ dB and F ₁ Bd	
Age at hypophysectomy, days	No. of mice	Mean change in weight, gm.	No. of mice	Mean change in weight, gm.
30-39	12	-1
40-49	9	-2	13	+5
50-59	18	-2	14	+5
60-79	8	-3	24	+3
80->	29	+1
Total	47	-2	80	+3

reached by mice bearing implants exceeded that of animals with none by more than 100 days. The numbers of mice are sufficiently large to show that these differences were not a result of mere chance. The conclusion to be drawn from this is that a subcutaneous implant prolongs the life of hypophysectomized mice considerably.

Table VII shows differences in body weight between hypophysectomized mice that were or were not implanted subcutaneously with another hypophysis.

The mice in which hypophysectomy was performed lost weight after the operation, especially if it took place at an older age; in other words the greater the weight on the day of operation the more weight was lost. In Groups III, IV, and V (B and dba) the loss was larger on the average than in the mice mentioned in Table VII. We observed the opposite in mice that had received implants of hypophyses, their average weight having increased distinctly, particularly in those operated upon when young. This is not surprising, however, because the mice operated

upon at an older age were already full grown at the time of operation.

The influence of the implants was perceptible on the ovaries also. In those of mice bearing implants we invariably found small groups of lutein cells whereas in those of mice without implants there were either no lutein cells or, if they were present, only a few were found.

Appendix. From our experiments it is clear that a hypophysis implanted subcutaneously does not exercise the gonadotropic influence required for an estrous cycle, and the question arose, therefore, whether in the mouse the hypophysis requires the connecting path to the brain provided by the stalk. To answer this question we cut through the stalk of the hypophysis in 5 F₁Od females, leaving the gland *in situ*. All these mice went through a series of complete estrous cycles after the operation. Eight months after operation they were killed healthy.

Owing to a technical error at autopsy the hypophysis was not found in 1 of the mice, but in the remaining 4 we were able to ascertain beyond any doubt that the stalk had been completely severed. The intermediate lobe showed considerable hypertrophy (Fig. 8), similar to that observed in rats by Desclin (1). From this experiment it follows that the path via the stalk of the hypophysis in mice is not essential for the occurrence of normal estrous cycles.

Because subcutaneous implantation of a hypophysis causes highly unphysiological conditions in the organ we thought it advisable to seek a better place for the implant. The most logical site seemed to be the natural location of the gland, at the base of the skull. Such an implantation was performed in 5 F₁Od females immediately after hypophysectomy, 2 of which afterwards showed estrus a number of times. Seven months later all were killed healthy, and at autopsy the implants were found lying at the base of the skull, but not in contact with the brain. They were slightly atrophic, but looked very much better than hypophyses implanted subcutaneously. Besides chromophobes they contained a moderate number of eosinophils and basophils.

Although this method indicated an important step forward in our technic, we had to abandon it because it was impossible to determine with certainty whether the hypophysis found was actually the implant, or remnants of the incompletely removed hypophysis of the mouse itself. We therefore tried implantation somewhere else in the same neighborhood. After a gap had been made in the ligamentum atlanto-occipitale, the hypophysis was deposited against the medulla oblongata. This operation was performed upon 5 F₁Od-males, but was discontinued because of operative difficulties.

As it is well known that in rats the hypophysis can be successfully transplanted into the eye, we tried to achieve the same result in the mouse. The operation was performed upon 14 F₁Od mice and was followed by hypophysectomy 1 or 2 days later. When we were about to test the function of the implanted hypophyses in the 10 animals upon which the operation seemed to have been performed with success, invasion of the Low Countries put an end to the experiment.

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The Heterologous Transplantation of Mouse Tumors Induced *in Vitro**

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In 1943 Earle reported the occurrence of morphological changes in cultures of mouse fibroblasts treated with 20-methylcholanthrene, and described the growth of tumors following injection of the cultures into mice of the parent strain (1, 2, 5). The histological appearance of the tumors, the capacity to invade, to metastasize, and to survive serial transfer left little question of their identity as sarcomas or of their autonomous nature. However, in view of the significance attached to their *in vitro* origin, a more precise definition of the growths with respect to the property of autonomy seemed desirable. Accordingly a series of heterologous transplantation experiments was carried out, and the results are reported in the present paper.

The experiments to be reported were conducted primarily to determine whether the tumors could be transferred to animals of alien species, but at the same time attempts were made to transfer to mice of different strains. Heterologous transplantation was readily accomplished, but it was found that the success of transfer to unrelated mice varied with the species or strain of the donor and an examination of this relationship formed a secondary object of the study.

MATERIALS AND METHODS

The tumors furnished by Dr. Earle included strains HW, D, J, L, N, and O. The strains H and D were derived from untreated control cultures, whereas the parent cells of strains J, L, N, and O had been treated with methylcholanthrene for 32, 111, 184, and 406 days respectively before transfer from culture to mice. The various strains had been carried by subinoculation in C3H mice of the Andervont stock for from 33 to 39 tumor generations previous to the present investigation.

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The tumors were made available through the courtesy of Dr. Wilton R. Earle, senior cytologist, National Cancer Institute, National Institute of Health, United States Public Health Service, Bethesda 14, Md.

Upon receipt in this laboratory the tumors were transferred to C3H mice of the Strong and Bar Harbor strains, and were maintained by serial transfer in these strains without difficulty. Other mouse strains used in transplantation experiments included the dba, C57 black, A albino, and Bagg albino. The dba and A strains were derived from Bar Harbor stock, while the C57 and Bagg albino strains were descendants of Bagg stock. Guinea pigs were used as alien hosts in heterologous transfers and, in several instances, the tumors were subsequently passed to rats. Transfer in mice was accomplished by the intramuscular implantation of tumor fragments, and the anterior chamber of the eye was used as a transplantation site in heterologous experiments. The technic of anterior chamber transfer has already been described in detail (4).

HETEROLOGOUS TRANSPLANTATION

Heterologous transplantation of all the various tumors, with the exception of those of the O strain, was readily effected. Transfer of the O strain tumor to the guinea pig's eye resulted in growth, but this occurred wholly in the manner of a tissue culture, without vascularization, and the intimate relationship between host and tumor characteristic of successful transplants was not observed. On the other hand the growth behavior of tumors of strains HW, D, J, L, and N was comparable with that of heterologous transplants of naturally occurring or chemically induced mouse sarcomas (3).

Tumors of the latter strains showed little variation in behavior in the pig's eye, and could not be differentiated one from the other by their manner of growth. Vascularization occurred early, and was usually apparent by the third day after transfer. Subsequent growth was rapid, and frequently the chamber was filled with tumor by the tenth day. Occasionally growth persisted for long periods of time, with rupture of the cornea and external protrusion, but in the majority of cases the tumors underwent regression after filling the chamber, a fate common to rapidly growing transplants of other tumors in this location.

The incidence of takes obtained with strains HW, D, J, L, and N is shown in Table I. All these tumors were transplanted serially. The majority were not carried beyond the second passage, but the transfer of strain J tumor was continued for 7 consecutive generations. The seventh generation transplants in the latter case grew rapidly and appeared healthy on histological examination, and there was no reason to believe that serial transfer could not have been continued indefinitely.

After the third anterior chamber generation fragments of the J strain tumor were transplanted intramuscularly in a series of 8 pigs. Takes occurred in 4 and the resulting tumors grew rapidly to form 4 cm. masses at the end of the third week. Two of the animals were held for further observation and in both instances regression occurred, so that no evidence of tumor remained a month after transfer.

Transfer from the guinea pig to the rat's eye was attempted with tissue obtained from the second genera-

resulted in approximately the same incidence of takes as had been obtained in the parent strain and, with continued serial transfer, sometimes exceeded it. Thus the first passage of the N strain tumor from C3H to dba mice resulted in 14 takes among 24 animals, the second passage in the dba strain gave rise to growth in all the 12 mice used, and the total incidence of takes in the next 6 serial generations in dba mice was 100 per cent as compared to 87 per cent in 6 coincident passages in C3H mice. Further, the growth rate and the metastatic rate were often greatest in a foreign mouse strain. In the series cited above, lung metastases were found in 2 of the dba mice, whereas none occurred in C3H animals. In general the tumors grew most rapidly, and reached the largest size, in Bagg albinos, and in a number of instances mice of this strain bore tumors approximately their body size at the end of the third week.

Whereas the second and subsequent transfers of the tumors in foreign mouse strains were consistently

TABLE I: RESULTS OF TRANSFER OF STRAIN HW, D, J, L, AND N TUMORS TO THE ANTERIOR CHAMBER OF THE GUINEA PIG'S EYE

Generation no.	HW		D		J		L		N	
	No. of pigs	No. of takes	No. of pigs	No. of takes	No. of pigs	No. of takes	No. of pigs	No. of takes	No. of pigs	No. of takes
1	20	17	8	7	10	8	25	23	30	22
2	9	9	6	4	10	10	20	20	18	16
3					15	11	15	14		
4					6	6	5	5		
5					5	5				
6					4	4				
7					5	5				

tion passage of the L and N strain tumors. A high percentage of takes occurred in both cases and the course of growth was comparable to that observed in the guinea pig.

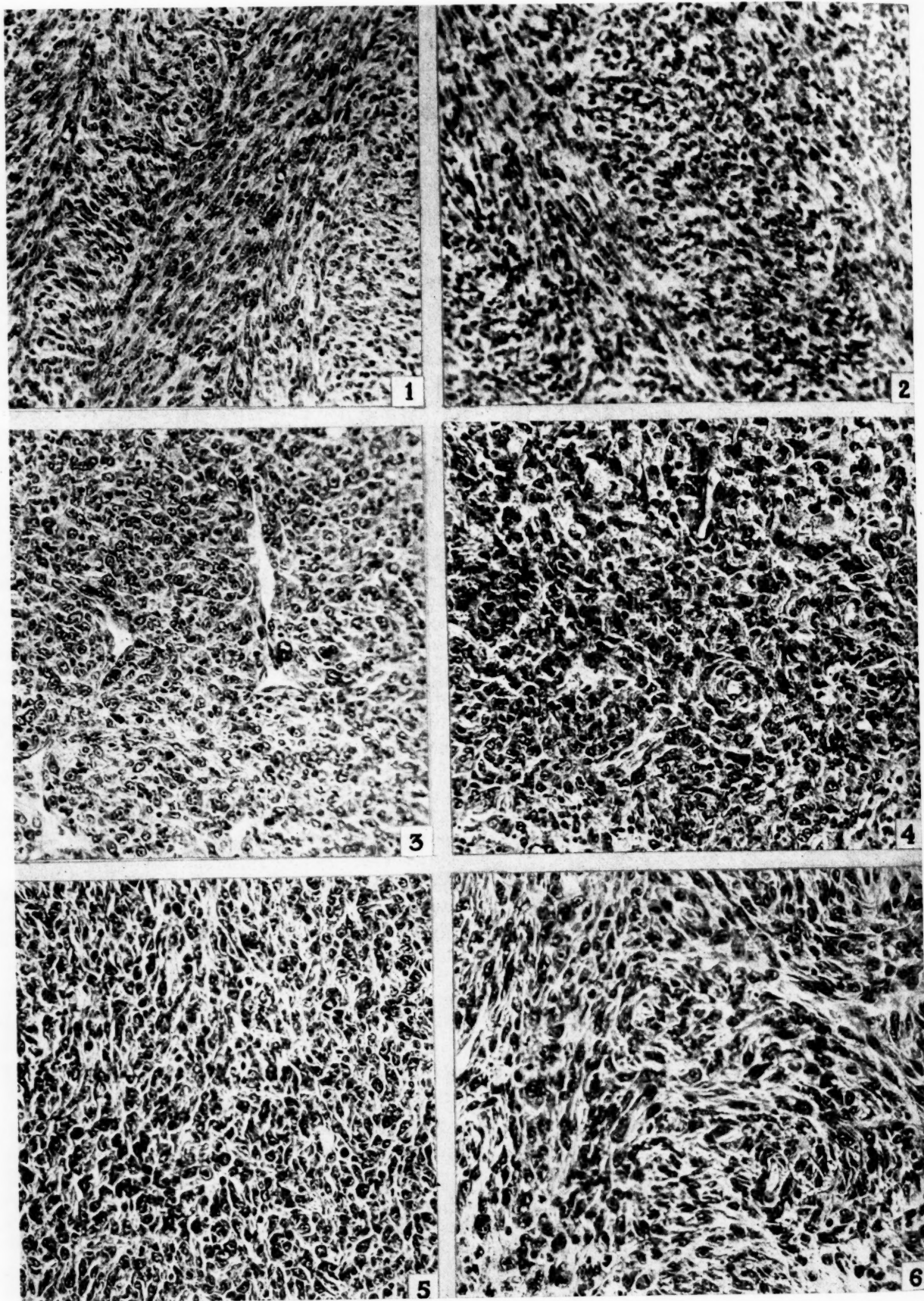
The transplants in the guinea pig and the rat were morphologically similar to the corresponding mouse tumors and displayed the same characteristics (Figs. 1 to 12). Sections of the O strain tumor in the pig's eye were obtained with considerable difficulty, and stained smears of the aqueous tumor proved more satisfactory for histological study. Such smears, with tumor cells lying free and unattached or in structureless masses without stroma or blood supply, resembled similar preparations of serous effusions from human cancer patients.

HOMOLOGOUS TRANSPLANTATION

Transfer of tumors of all the different strains to unrelated mice was successfully effected. The percentage of takes in the first generation was often low, but the second passage in the new mouse strain

successful, a wide variation in the incidence of takes characterized the first passages. In Table II the results of first generation transfers have been arranged in such manner as to show the participation of the strain of the donor, recipient, and tumor in this variation. Examination of the data demonstrates that the tumor strain and the strain of the recipient mouse influenced the incidence of takes, but that a major factor in this determination was the strain of the donor.

The first attempt to transfer the tumors to foreign mouse strains was made with neoplasms from C3H mice and the results were generally poor, with only occasional growths except in the dba strain, where the incidence of takes was relatively high. In a later attempt to transfer to the seemingly refractory strains, tumor tissue growing in the dba mice was used as an inoculum and takes occurred with a much higher frequency than when C3H tissue was employed. Finally the experiment was extended to the transfer of tumors grown in guinea pig eyes, and the percentage of takes far exceeded that obtained in either of the two previous cases.



FIGS. 1-6

TABLE II: RESULTS OF FIRST GENERATION TRANSFERS IN FOREIGN MOUSE STRAINS *

Tumor	Donor	C3H			dba			Recipient C57			Bagg Albino			A Albino		
		No.	Takes	%	No.	Takes	%	No.	Takes	%	No.	Takes	%	No.	Takes	%
HW	C3H † dba	5	5	100	38	8	21.0	23	1	4.3	14	7	50	11	2	18.1
								17	4	23.5	9	6	66.6	10	6	60
O	C3H dba	8	6	75	16	7	43.7	26	0	0	10	0	0	7	3	42.8
								30	4	13.3	15	2	13.3	15	6	40.0
N	C3H	5	5	100	24	14	58.3	19	0	0	19	2	10.5	19	2	10.5
	dba							21	3	14.2	25	4	16	19	9	47.3
	G. P.							17	11	64.7	10	6	60	10	10	100
L	C3H	5	3	60	32	3	9.3	25	0	0	15	0	0	15	0	0
	dba							6	0	0	5	0	0			
	G. P.							26	13	50	16	9	56.2	11	5	45.4
Combined	C3H	23	19	82.6	110	32	29.0	93	1	1.0	58	9	15.5	52	7	13.4
	dba							74	11	14.8	54	12	22.2	44	21	47.7
	G. P.							43	24	55.8	26	15	57.6	21	15	71.4

* Transplantation of the H and O tumors was carried out with tissue derived from C3H and dba hosts, but experiments with the N and L tumors were extended to include transfers from guinea pig hosts.

† In the transfer C3H to C3H the donor was of Andervont stock and the recipient of Bar Harbor stock. In other transfers the C3H donor was of Bar Harbor stock.

DISCUSSION

An evaluation of the significance of Earle's *in vitro* experiments rests on an identification of the induced tumors with the naturally occurring sarcomas. Histologically such an identification is complete, and the present experiments were conducted to determine the extent of biological relationship. The comparison was based on the biological attribute of autonomy, a property common to all naturally occurring or chemically induced sarcomas tested in this laboratory and expressed by the ability to survive and to grow in animals of alien species.

The behavior of Earle's tumors on heterologous transplantation has been described, and differs in no essential respect from that of other sarcomas. The failure of the O tumor to elicit a vascular response in the new host is not without precedent, having been observed in the case of other anaplastic growths. Thus during the course of consecutive biopsy study of a developing rabbit tumor a point is sometimes reached when transfer to the eyes of other rabbits or of guinea pigs results in a tissue culture type of growth, despite the fact that earlier transfers gave rise to tumors

with typical stromal-parenchymal relationships. Such behavior, always associated with the disappearance of identifying morphological characteristics in the primary tumor, suggests that a loss of the ability to provoke a vascular reaction accompanies anaplastic development. The phenomenon is of considerable interest in view of the natural history of anaplastic tumors and is the subject of continued study. The point to be emphasized in the present connection is the identity of Earle's tumors with other sarcomas. The fundamental significance of his experiments, or the implications to be derived from the fact of *in vitro* carcinogenesis, need not be discussed here.

The results of transplantation of the tumors to foreign mouse strains are of interest from a different viewpoint and require some further comment. It was noted that the strain of the donor mouse was of great influence. Thus a tumor grown in C3H mice was less readily transplantable than the same tumor grown in dba mice or in guinea pigs. Both the range of transplantability and the incidence of takes increased after residence in the foreign hosts and reached a maximum after growth in guinea pigs. Such varia-

DESCRIPTION OF FIGURES 1 TO 6

All sections stained with hematoxylin and eosin.

All photographs magnified $\times 250$.

FIG. 1.—Transplant of strain HW tumor in muscle of C3H mouse.

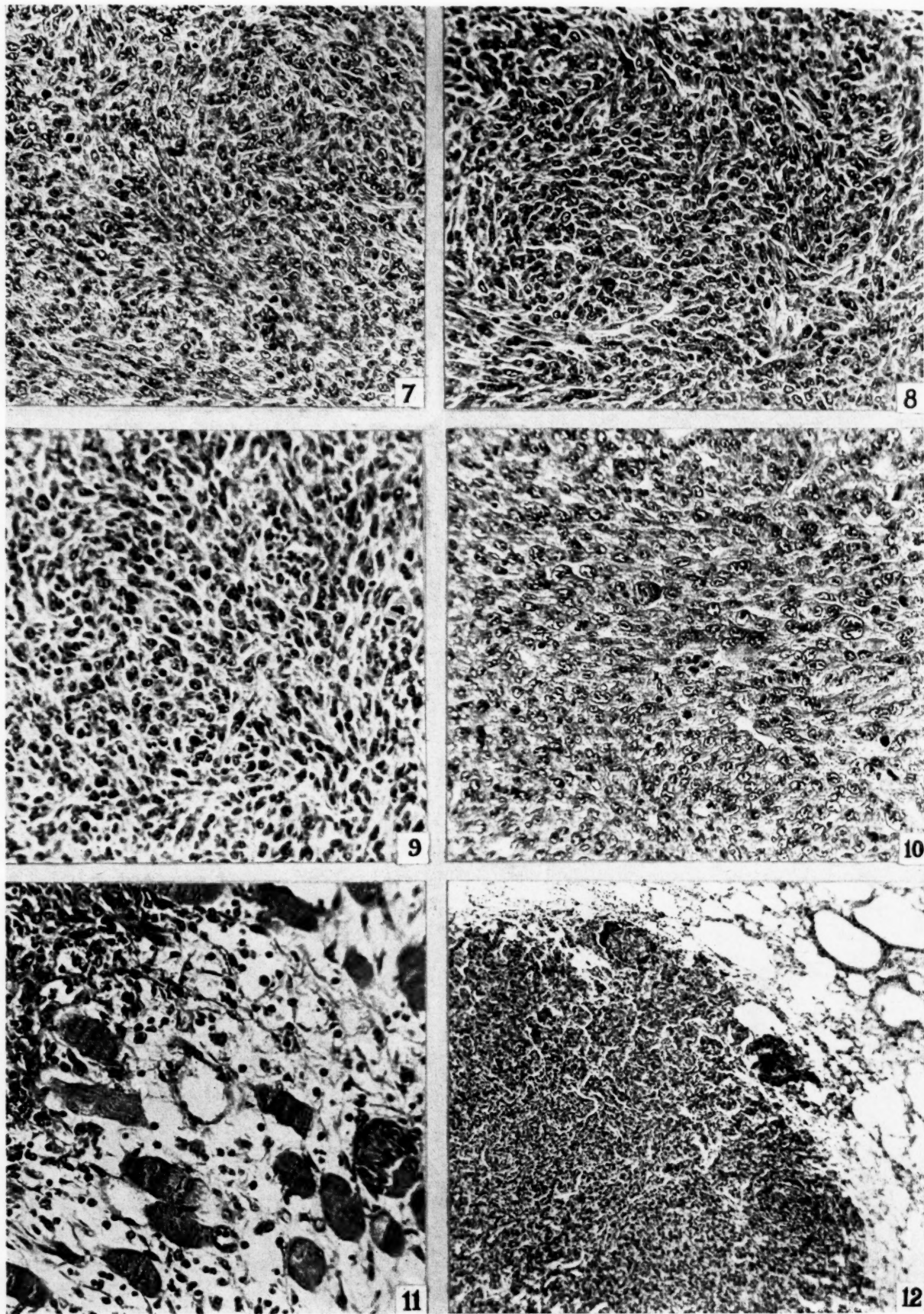
FIG. 2.—Transplant of strain HW tumor in anterior chamber of guinea pig's eye.

FIG. 3.—Transplant of strain D tumor in muscle of C3H mouse.

FIG. 4.—Transplant of strain D tumor in anterior chamber of guinea pig's eye.

FIG. 5.—Transplant of strain J tumor in muscle of C3H mouse.

FIG. 6.—Transplant of strain J tumor in anterior chamber of guinea pig's eye.



FIGS. 7-12.

tion in behavior is suggestive of a change in the essential nature of the tumor incident to growth in the new environment, but further consideration based on the varying composition of the tumor in different strains and species indicates another explanation.

The parenchyma of a transplanted tumor persists on transfer and is not a product of the new host. Unlike the stroma, it represents a continuous proliferation of the original cancer cell and there is no reason to believe that its transplantability should be different from that of the parent cell. Conversely, the stroma of a transplanted tumor is a product of the new host and is replaced at each transfer. Thus when a tumor is transferred from a C3H to a dba mouse the C3H stroma dies and the transplant is provided with a framework of normal connective tissue and blood vessels by the dba mouse. Transfer of the tumor from mouse to guinea pigs, in like manner, is associated with destruction of the mouse stroma and replacement with guinea pig stroma.

It is apparent, therefore, that transplants of the same tumor growing in C3H and dba mice may be identical in parenchyma but differ in stroma. Accordingly, it is suggested that the transplantability of the growths may vary in consequence of their stromal composition rather than as a result of parenchymal modification. The connective tissue of C3H and dba mice, comprising the stroma of their tumors, may differ in the ability to induce foreign body reactions when introduced into other mouse strains. In such a case the fate of the parenchyma would be determined to a large extent by the intensity of the ensuing inflammatory reaction, and survival and growth or death and destruction would depend on the relative compatibility of the connective tissue of donor and recipient.

An objection to this suggestion might be based on the fact that a much higher percentage of takes was obtained in guinea pigs than in foreign mouse strains despite the discrepancy in connective tissue relationships; the differences in the first instance being of a species order and of only a strain order in the second. It should be emphasized in this connection that the anterior chamber of the eye was utilized as an inocu-

lation site in guinea pig transfers, whereas the subcutaneous tissues were used in mouse transfers. The anterior chamber differs from other bodily regions in respect to the rapidity and intensity of foreign body reactions and, by virtue of this difference, offers a unique site for the growth of tumors of other species. The reaction of the iris to a foreign implant may be delayed for 3 or more days, and in the interim the alien connective tissue dies and disintegrates while the parenchyma proliferates, imbibing nutrient from the surrounding fluid in the manner of a tissue culture. As a consequence the eventual inflammatory response is much less intense than that following immediately after the introduction of foreign tissue into the subcutaneous tissues, and results in vascularization rather than in destruction of the parenchyma. In actuality, therefore, the high incidence of takes in the guinea pig's eye tends to support rather than to negate the suggestion that connective tissue relationships rather than parenchymal modification may form the basis of the observed variation in transplantability.

Further supportive evidence may be derived from the results of transfer of tissue grown in guinea pigs to mice of the various strains. The stroma of such tumors although guinea pig in identity is present in minimal amounts and consists only of thin-walled blood vessels. Moreover, the manner of growth in the eye allows removal of most of the transplant without the inclusion of other guinea pig tissue. The absence of contaminating adult tissue, together with the paucity of stroma, is in contrast to the large stromal component and inseparable surrounding tissues of subcutaneous growths in mice, and may be the determining factor in the observed variation in behavior.

The point of interest suggested by these experiments is that the stroma of a tumor may influence its transplantability. It has been generally thought that the parenchyma or essential cellular content alone determines its behavior on transfer, and that variations in transplantability of the order described stem from mutations in parenchymal cells. Nevertheless, the indications of the present experiments are sufficiently strong to warrant further investigation, and pertinent studies are in progress.

DESCRIPTION OF FIGURES 7 TO 12

All sections stained with hematoxylin and eosin

All photographs magnified $\times 250$.

FIG. 7.—Transplant of strain L tumor in muscle of C3H mouse.

FIG. 8.—Transplant of strain L tumor in anterior chamber of guinea pig's eye.

FIG. 9.—Transplant of strain N tumor in muscle of C3H mouse.

FIG. 10.—Transplant of strain N tumor in anterior chamber of guinea pig's eye.

FIG. 11.—Transplant of strain N tumor in muscle of dba mouse. Note true muscle invasion.

FIG. 12.—Metastasis of strain N tumor in lung of dba mouse.

SUMMARY

The tumors induced *in vitro* by Earle have been successfully transplanted to guinea pigs and to mice of foreign strains. The ability to survive and to grow in animals of alien species identifies the tumors with the chemically induced and naturally occurring sarcomas, and adds further significance to their mode of origin.

The success of transfer of the tumors to unrelated mice varied with the strain or species of the donor, and an examination of this relationship suggested that the stromal component of the tumor was concerned in the variation.

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Radio Isotopes from the Manhattan Project

A detailed announcement on the availability and procurement of pile-produced radioisotopes from the Manhattan Project appeared in the June 14 issue of *Science*. (103:697-705. 1946)

Tables are included giving pertinent data on the characteristics and the quantities that may be made available of approximately 100 isotopes and isotopic mixtures. For practical reasons isotopes with a half-life less than 12 hours are not considered for distribution. Most of the isotopes are produced by fission or (n, γ) processes. Only four isotopes are produced by the (n,p) process with sufficient yield for distribution. Other processes are either not sufficiently productive or do not occur.

The article emphasizes that (1) present piles were not designed for tracer and therapeutic isotope production, (2) waste plutonium process solutions are not a feasible source for separated fission isotopes, (3) routine production methods and facilities are not yet developed for most isotopes, (4) isotopes which can now be made available are only experimental lots resulting from research and development proceedings,

(5) technical problems involved in the irradiation and processing of essential materials has been and will continue to be responsible for the delay in making certain isotopes available by routine production.

Allocation and distribution will be effected on the basis of the general policies, as well as on recommendations regarding specific applications, made by well qualified advisory groups nominated for Manhattan District appointment by the National Academy of Sciences. Charges will be made for materials and services on the basis of "out-of-pocket" operational expenses to the Government necessitated by the non-project production and service program. Costs for construction or rental of major plant facilities and expenses of research and development on isotope production will be assumed by the Project.

All correspondence concerning radioisotope procurement should be addressed to the Isotopes Branch, Research Division, Manhattan District, P. O. Box E, Oak Ridge, Tennessee. Reference to the original article for pertinent details is recommended, however, before instituting inquiries or requests.

The Production of a Carcinogenic Agent in the Degradation of Cholesterol to Progesterone*

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INTRODUCTION

Ever since the carcinogenic hydrocarbons and the steroid hormones, which may influence carcinogenesis, have been shown to be structurally related to cholesterol, the animal sterol par excellence, speculation has been active concerning both the endogenous and exogenous conversion of these compounds, one to another, as a possible cause of cancer (7). By means of degradative oxidation (and in some cases reduction) cholesterol may be converted to the steroid hormones in the test tube, and cyclization of the aliphatic side chain could give rise to the ring skeleton of the carcinogenic hydrocarbons. Carcinogens have been isolated from the liver (5) and cholesterol changed structurally by heat has been held responsible for gastric carcinoma (11). Although literally hundreds of steroid compounds have been prepared by the organic chemist, there are still wide gaps in the series of probable compounds that could form as intermediary steps in the path of a degradation. The possibility of these compounds serving as carcinogens or being more readily converted endogenously to carcinogens is not known to have been realized.

The present report is an outgrowth of an accidental discovery, which is considered a valuable clue to the ultimate solution of the problem.

In 1941, when this problem was begun, it was known that progesterone, like the estrogens, is intimately connected with the mechanism regulating the development and maintenance of the mammary gland; however, in contrast to the estrogens its influence on carcinogenesis had not been proved (1). Since the Marsh-Buffalo strain of mice is a high cancer strain resistant to the influence of estrogens (2), an investigation of the possible role of progesterone in carcinogenesis in this strain was prompted. A chemically crude but accurately standardized synthetic preparation was used in the initial experiments, and it proved to have carcinogenic properties. It was then necessary to repeat the experiment with crystalline progesterone,

which was the biologically active ingredient in the original experiment; crystalline cholesterol, which was the starting material; and crystalline cholestenone, which was a known contaminating substance. These all proved to be noncarcinogenic. One had therefore the choice in pursuing this problem farther either of attempting to isolate the carcinogenic compound from the reaction product or of testing out known compounds that would be expected to form as links in the degradative chain on the basis of the chemical procedure. Both lines of attack are being pursued in this laboratory. Our original experiments are presented, in view of the rapid strides being made in steroid chemistry and the possibility that a compound that would fit into the chemical scheme yielding the carcinogenic effect might be isolated by some other investigator. Interest is also attached to the original purpose of the first experiment; namely, the role of progesterone in the development of mammary cancer.

EXPERIMENTAL

Plan of animal experimentation.—Virgin Marsh-Buffalo female mice were employed in 3 groups of long-term experiments. Each group contained a division of intact control mice, which received injections of sesame oil equal to the amount serving as a vehicle for the steroid given the other 2 divisions of the group. The mice of 1 division receiving the steroid were castrated at 23 days of age; those of the other remained intact. The injections in all cases were dorsal, and were given subcutaneously as far as possible from the mammary glands. Details of dosage follow.

GROUP I

Experiment 1.—Thirty-eight control mice received 0.08 cc. of sesame oil per mouse weekly; a total of 1.7 cc. per mouse was administered from the second to the ninth month of age.

Experiment 2.—Thirty-eight intact mice received a total of 10 units of crude progesterone per mouse.

Experiment 3.—Thirty-eight castrated mice received a total of 10 units of crude progesterone per mouse. (A biologic unit is 1 mgm., so that each mouse received in addition about 20 mgm. of supposedly inert material.)

*Aided by grants from The International Cancer Research Foundation, and from Mr. and Mrs. Herman F. Pelphrey. Presented at the Second Mexican Congress of Cancer in Guadalajara, Mexico, February 3, 1946.

GROUP II

Experiment 4.—Thirty-five control mice received 0.08 cc. of sesame oil per mouse weekly; a total of 1.7 cc. per mouse was administered from the second to the eighth month of age.

Experiment 5.—Thirty-five intact mice received a total of 20 mgm. of crystalline cholesterol per mouse.

*Experiment 6.*¹—Thirty-five castrated mice received a total of 10 mgm. of crystalline progesterone per mouse.

GROUP III

Experiment 7.—Thirty-four control mice received 0.1 cc. of sesame oil per mouse weekly; a total of 1.6 cc. per mouse was administered from the second to the seventh month of age.

*Experiment 8.*²—Thirty-four intact mice received a total of 19 mgm. of cholestenone per mouse.

*Experiment 9.*²—Thirty-five castrated mice received a total of 19 mgm. of cholestenone per mouse.

In addition to the long-term experiments described above, the effect of estrone and progesterone alone and in combination upon the development of the mammary gland of virgin Marsh-Buffalo mice was studied.

SOURCE AND PURITY OF STEROIDS

The progesterone used in the first experiments (2 and 3) was especially made for us at our request, in the laboratories of Eli Lilly and Company, by the oxidation of cholesterol according to the method of Spielman and Meyer (12). No effort was made at further purification beyond that outlined in the method. The product contained about 60 per cent by weight of so-called inert material. In the preparation of progesterone by this method cholestenone is the main by-product, and has been tested in our present studies. Some androgenic material also is formed in a side reaction, and this is undoubtedly androstenedione; but the amount present in the sample used by us was negligible because of partial purification. One unit of this product given to castrated rats produced no effect upon seminal vesicles and prostate, and it required 10 units to double the weight of the former.

The cholesterol given in Experiment 5 was prepared from human gallstones by cold alcohol extraction, and had been recrystallized 3 times. The

Lifschütz reaction, a very sensitive test for oxidative impurities, was negative. Spectrographic analyses of this cholesterol gave a different curve from the so-called C. P. preparations available on the market. Some of these have probably suffered denaturation.

The steroid in each instance was dispersed in sesame oil by addition of an ethanol solution of the steroid. The alcohol was evaporated by heating the oil at the temperature of the water bath.

RESULTS OF LONG-TERM EXPERIMENTS

GROUP I

Tumors of mammary gland.—Progesterone had no influence upon mammary tumor formation in the intact mice (Experiment 2). While the cumulative incidence was 16 per cent lower than the control incidence at the 18th month of age the difference is not significant; fewer mice were available in the treated group for mammary tumor development because of the development of more lymphoid tumors and of malignant growths at the site of injection, and because of a higher death rate from causes not related to tumor formation. The 11 per cent incidence of mammary tumors in the castrated mice (Experiment 3) would appear to be significant, as Cori found no mammary tumors in Buffalo mice castrated at the same age (4). The uteri of the castrated mice that developed mammary tumors were rudimentary threads.

Lymphoid tumors.—The 21 per cent incidence of lymphoid tumors in the castrated mice (Experiment 3) would be significantly greater than the 3 per cent incidence found in the controls if other factors removing mice from the experiment had been balanced. However, since there were more than twice as many mice available for lymphoid tumor development in the castrated group as in the controls at the 16th month of age, the significance of the uncorrected accumulated incidences may be questioned.

Nonmalignant tumors.—A striking result of the experiment was the high incidence (60 per cent in Experiment 3, 34 per cent in Experiment 2) of plaque-like tumors at the site of injection of progesterone in sesame oil, and a complete absence of these tumors in the controls, which received only sesame oil. These tumors were ochre colored, oily in appearance, and rather friable; their average weight at autopsy was 400 mgm.; their range, from 100 to 1,600 mgm. On preparation of paraffin sections the greater portion disintegrated, indicating a high content of debris. The walls of these tumors were characterized by sheaths of fibrous tissue in which were incorporated vacuoles of various sizes; areas of myxomatous degeneration, of

¹ The crystalline progesterone administered in Experiment 6 was supplied by Parke, Davis and Company and by the Schering Corporation.

² The cholestenone used in Experiments 8 and 9 was supplied by the Abbott Laboratories and by the Schering Corporation (m.p. 79° to 81° C.).

necrosis and hemorrhage, foreign body giant cells, and nests and rings of fibroblasts were not uncommon. An analysis of these growths follows.

Analyses of oleomas.—At autopsy they were dissected free from the surrounding tissues, weighed, and preserved in 95 per cent ethanol. Approximately 7 gm. of this material was collected in each experiment, ground in a glass mortar, and extracted in the cold with 200 cc. of 95 per cent ethanol. The residue was taken up in 50 per cent ethanol and extracted twice with petroleum ether. An aliquot of all extracts was evaporated to dryness at room temperature and re-extracted, first with petroleum ether then with chloroform.

	Lipids, per cent	Cholesterol, per cent
Injected normal	8.5	0.33
Injected ovariectomized	10.2	0.41

Malignant tumors.—One control mouse developed a lymphosarcoma beneath an area of skin that may have been in contact with the sesame oil. In contrast 11 per cent of the mice in Experiment 2 and 21 per cent of those in Experiment 3 developed malignant tumors at the site of injection. In Experiment 3 there were 7 fibrosarcomas and 1 lymphosarcoma. One fibrosarcoma was a mixed tumor, with an area of adenocarcinoma. In Experiment 2 there were 3 fibrosarcomas and 2 plaques, the walls of which showed changes indicative of malignancy.

GROUP II

Tumors of the mammary gland.—Cholesterol given to intact mice had no influence upon the development of mammary tumors (Experiment 5). Crystalline progesterone given to mice ovariectomized at 23 days of age produced no tumors of the mammary gland.

Lymphoid tumors.—No effect upon lymphoid tumor formation was indicated.

Nonmalignant tumors.—In contrast to Experiment 2 and 3, plaques of inert material did not form at the site of injection in Experiments 5 and 6. There were, however, a number of deposits of oil, which were enclosed in a membrane. Sections of the membranes of 3 mice treated with cholesterol showed round cell infiltration, foreign body giant cells, slits, and spaces that had probably contained sesame oil. The analyses of the cholesterol content of the oil in these cysts follow; it should be noted, however, that the test for cholesterol, which was the conventional Lieberman reaction, is not specific for this compound but is given by the phytosterols also, and that the

sesame oil contained an appreciable amount of the substance giving the test.

Oil cysts of	Age of mice, months	Total sterol, per cent	Sterol esters, per cent	Ratio
3 Control mice (Exper. 4)	15	2.53		
1 Control mouse (Exper. 4)	15	2.38	1.78	.75
1 Control mouse (Exper. 4)	15	2.6	1.36	.52
Blank for sesame oil		0.80		
1 Cholesterol-treated mouse (Exper. 5)	15	5.24		
1 Cholesterol-treated mouse (Exper. 5)	15	4.23	3.08	.73
1 Cholesterol-treated mouse (Exper. 5)	17	7.27	2.89	.40
1 Progesterone-treated mouse (Exper. 6)	17	2.80	1.59	.57
4 Progesterone-treated mice (Exper. 6)	17	3.95	2.29	.58

Malignant tumors.—There were no malignant tumors produced at the site of injection in Experiments 4, 5, or 6.

GROUP III

Tumors of the mammary gland.—In the intact mice (Experiment 8) that received cholestenone the cumulative incidence of mammary tumors was increased over that observed in controls (Experiment 7) by 20 per cent at the 12th and 13th months of age. The difference is twice the standard deviation of the mean, and would be considered significant. The final course of mammary tumor development was not different from that of the controls. The ovariectomized mice that received cholestenone did not develop mammary tumors.

Lymphoid tumors.—The increase in lymphoid tumor formation occurring at the end of the experiment in the ovariectomized mice is probably not significant, since none developed mammary tumors and more mice were available for lymphoid tumor formation.

Nonmalignant tumors.—Fourteen per cent of the controls (Experiment 7), 56 per cent of the treated intact mice (Experiment 8), and 47 per cent of the treated ovariectomized mice (Experiment 9) developed the ochre colored plaques at the site of injection. These have been described under Experiments 2 and 3 as consisting mainly of inert material.

Local malignant tumors.—One mouse in each of Experiments 7, 8, and 9 developed a fibrosarcoma at the site of injection.

TABLE I: CUMULATIVE INCIDENCE OF TUMOR FORMATION AND DEATH DUE TO OTHER CAUSES IN MARSH-BUFFALO MICE THAT RECEIVED A SERIES OF CHEMICALLY RELATED STEROIDS GIVEN AS PERCENTAGE OF CASES

Month	Exper. 1 Control (38 mice)				Exper. 2 Crude progesterone. Treated, intact (38 mice)				Exper. 3 Crude progesterone. Treated, ovariectomized (38 mice)			
	Ad. ca. mammary gland	Lympho- sarc.	Local cancer	Other causes	Ad. ca. breast	Lympho- sarc.	Local cancer	Other causes	Ad. ca. breast	Lympho- sarc.	Local cancer	Other causes
7				3								
8				3	3			6				3
9	8			3	6			6				3
10	8			3	13	6	6	6	3		8	3
11	11			6	13	6	6	6	6	3	11	3
12	18			6	18	8	8	6	6	3	13	3
13	34			6	34	11	11	6	8	6	18	3
14	42	3		6	42	11	14	13	8	8	18	3
15	60	3	3	6	48	11	14	13	11	11	18	3
16	63	3	3	6	48	11	14	18	11	13	18	3
17	63	3	3	6	48	11	14	21	11	18	21	3
18	66	3	3	6	50	11	14	21	11	21	21	3

Month	Exper. 4 Control (35 mice)				Exper. 5 Cholesterol. Treated, intact (35 mice)				Exper. 6 Crystalline progesterone. Treated, ovariectomized (35 mice)			
	Ad. ca. mammary gland	Lympho- sarc.	Local cancer	Other causes	Ad. ca. breast	Lympho- sarc.	Local cancer	Other causes	Ad. ca. breast	Lympho- sarc.	Local cancer	Other causes
7								6				3
8	3				3	3		6				3
9	6			3	17	6		6				3
10	12	3		6	20	8		6				3
11	24	6		8	26	12		6				3
12	36	8		8	34	12		6				3
13	39	12		17	46	12		6				3
14	41	12		17	49	12		6	6			3
15	44	12		24	57	15		6	8			3
16	47	12		26	57	15		6	12			3
17	53	12		26	57	15		6	15			3
18												

Month	Exper. 7 Control (34 mice)				Exper. 8 Cholestenone. Treated, intact (34 mice)				Exper. 9 Cholestenone. Treated, ovariectomized (34 mice)			
	Ad. ca. mammary gland	Lympho- sarc.	Local cancer	Other causes	Ad. ca. breast	Lympho- sarc.	Local cancer	Other causes	Ad. ca. breast	Lympho- sarc.	Local cancer	Other causes
7		3										
8		3			6				3			
9	3	3			9				3			
10	3	3			12				3			
11	6	3			23	3			3			3
12	12	3			32	3			3			3
13	15	3		3	38	3			3			9
14	38	3		3	44	6			12			9
15	47	3		6	56	9			17			9
16	56	3	3	9	59	9			26			9
17	62	3	3	9	61	9			26			9
18	62	12	3	9	68	12	3		32			12

Influence of estrone and progesterone on mammary gland development.—Twenty-four virgin female Marsh-Buffalo mice were segregated in 4 experimental groups at the age of 6 months. One group, receiving only injections of sesame oil, served as a control. One group received weekly injections of estrone in oil, another received progesterone in oil, and a third received a combination of these two

hormones. Injections were made subcutaneously as in the long-term experiments. The period of treatment covered 5 weeks, and the total amount of hormone administered per mouse was 500 units of estrone and 3 (3 mgm.) of progesterone. Whole mounts of the lower mammary gland of each mouse were prepared and a 0, 1, 2, 3, 4 classification was made on the basis of 3 objective measures: number of ducts, width

of ducts, and number of alveoli. The results are given in Table II. The results show that: (a) the

TABLE II: INFLUENCE OF ESTRONE, PROGESTERONE, AND THEIR COMBINATION UPON MAMMARY GLAND DEVELOPMENT IN THE SIX MONTH VIRGIN MARSH-BUFFALO MOUSE

Treatment	Histology of Mammary Gland 0, 1, 2, 3, 4 Classification		
	No. of ducts	Width of ducts	No. of alveoli
Control	2.7 \pm 0.5	2.4 \pm 0.5	2.0 \pm 0.6
Estrone, 500 u. per mouse	2.7 \pm 0.3	1.9 \pm 0.3	2.2 \pm 0.5
Progesterone, 3 mgm. per mouse	2.5 \pm 0.3	2.2 \pm 0.4	2.7 \pm 0.6
Estrone and progesterone as above	3.2 \pm 0.3	1.7 \pm 0.3	2.5 \pm 0.4

7 months old female of the Marsh-Buffalo strain may show no alveolar development excepting terminal buds (confirming previous work); (b) neither estrone, progesterone, nor a combination of the two produced any demonstrable effect upon ductal or alveolar development.

DISCUSSION

Progesterone and carcinogenesis.—Heiman (8) has shown that in the RIII strain progesterone in the dose range used in our experiments has a pronounced inhibitory effect upon the incidence of mammary tumors; none appeared in castrated females of this strain that had received the hormone. Our results, therefore, agree with his, in that progesterone is noncarcinogenic, but the inhibitory effect shown for strain RIII is obviously not encountered with Marsh-Buffalo mice, which have previously shown (2, 3) a notable resistance to the carcinogenic effect of estrogens. This resistance is substantiated by their failure to respond to estrone and progesterone both alone and in combination with development of the mammary glands, as recorded in the present experiments. Heiman's interpretation of his results is that the hormones employed probably reduced the pituitary gonadotropic fraction, and that this deficiency was in turn followed by a suppression of ovarian secretion. In this respect it is interesting to note that we were able to suppress ovarian secretion, and thus reduce the incidence of cancer, by long-continued administration of mare serum and sheep pituitary gonadotropins, but not by the administration of human chorionic gonadotropin (2).

Sterol exchange from the sesame oil depot.—The analyses of oil cysts from mice that had received sesame oil without steroid showed a total cholesterol content, estimated by the Lieberman reaction, of 2.4 to 2.6 per cent. The blank for the sesame oil was 0.8 per cent, so that the accumulation of cholesterol-like ma-

terial from the body fluids is indicated. The high ester content is characteristic of blood. In the mice that received cholesterol in a concentration of 1.2 per cent, the cholesterol content of the oil cysts varied from 4.2 to 7.3 per cent, indicating that the presence of cholesterol increased the deposition of extra cyst cholesterol above that which formed in the cholesterol-free oil. The influence of crystalline progesterone is intermediate. These analyses show rather conclusively that in the oil cyst there can be both a release and an accumulation of steroid; in other words, the cyst is in equilibrium with the steroid system of the body fluids. These analyses should be contrasted with those of the oleomas that formed after the injection of sesame oil containing impure progesterone or cholestenone. In these the cholesterol content was only 0.3 to 0.4 per cent and the total lipid content 8.5 to 10.2 per cent. The ratio of cholesterol to total lipid is of the same order, so that in the oleoma water, protein, and electrolytes have to a large extent replaced fat. One would be tempted to ascribe the formation of the nonmalignant plaque to the presence of cholestenone, since the plaques formed to considerable extent only in those experiments in which pure cholestenone was administered or was present as an impurity. There is no evidence that cholestenone contributed to the formation of malignant tumors at the site of injection. Kirby (9), in his experiments on feeding derivatives of heated cholesterol, states that cholestenone is noncarcinogenic. However, it should be noted that in Experiment 8, in which intact mice received pure cholestenone, the onset of mammary tumors was hastened, statistical analyses of the data indicating that the observation was significant.

Local tumors.—The production of cysts following the intramuscular injection of vegetable oils is well known (10). It has been shown (6) that estrone is resorbed from a sesame oil depot in from 3 to 9 days. Though it is common practice in many research laboratories to aspirate the oil cysts that form in the course of an experiment we have not done so; it is apparent that the formation of a cyst from a subcutaneous injection gives opportunity for the local development of the carcinogenic process depending on the steroid content, and thus offers a valuable tool in the study of cancer. In the large series of experiments in which we have injected estrogens in sesame oil, the formation of local skin tumors (usually fibrosarcoma) was never above the normal incidence in females. In comparing Experiments 3, 6, and 9, which are concerned with ovariectomized mice, it is revealed that in Experiment 3, 21 per cent of the mice developed malignant tumors at the injection site (which figure does not include the 11 per cent that developed adenocarcinomas), while no malignant

tumors whatsoever appeared here in Experiments 6 and 9. The mice of Experiments 6 and 9 received crystalline progesterone or crystalline cholestenone, and the crude progesterone of Experiment 3 is thus proved to contain a carcinogenic element, since 21 per cent is 3 times the standard deviation of the mean. If the 11 per cent incidence of adenocarcinoma is added, the ratio of 32 to 0 per cent becomes formidable. There is every reason to believe that the adenocarcinoma was a local effect, since histological study indicated that the mammary gland areas came in contact with the developing oil plaques, and the uteri remained infantile, showing the absence of estrogens. It should be stressed that there is a tendency in female Marsh-Buffalo mice to form tumors of the skin (usually fibrosarcoma), and the appearance of 1 fibrosarcoma each in Experiments 1, 2, and 7, and none in Experiments 4, 5, and 6, giving an incidence of 3 out of 245 mice, or 1.2 per cent, is normal. In previous experiments 2 fibrosarcomas developed in 176 female mice (1.1 per cent) injected with estradiol in sesame oil. Of 95 castrated males, 2 developed a fibrosarcoma (2.2 per cent). These data are compiled to the age of 17 to 18 months, when the experiments were terminated. In previous experiments male mice observed for 20 months or more developed a higher incidence of skin tumors; namely, 10 per cent in 30 control mice, and 23 per cent in 30 males that had received estrone. Steiner and his group (13) found that 61 mice of heterogeneous stock injected with sesame oil as a vehicle for other substances failed to develop skin tumors. On the other hand, 3 of 9 mice developed sarcoma when injected with sesame oil heated to 350° C.

Endogenous production of carcinogens.—The product that exerted a local carcinogenic effect in our experiments was the crude progesterone made by the method of Spielman and Meyer from cholesterol by oxidation of the dibromide of cholesterol in benzene solution with aqueous permanganate. The relation of the reaction products is given in Fig. 1. Cholesterol, cholestenone, and progesterone have been eliminated as carcinogenic agents by the results of the present experiments. Androstenedione is eliminated by its absence in any amount exceeding a trace in the material used. Suspicion rests, therefore, on the large series of possible intermediate compounds that theoretically could be formed in either of the paths of degradation. The progress of the research is reported to this point: Oxidation and reduction of cholesterol dibromide according to the procedure of Spielman and Meyer gave a product that was carcinogenic. It is considered worth while to pursue the problem farther, since the reactions in the Spielman-Meyer procedure are not unlike those that conceivably could

occur in the organism. The accidental discovery that this process leads to a carcinogen is considered a valuable clue in the effort to banish the spectre that exists at present: The question whether or not carcinogens are endogenously produced from cholesterol.

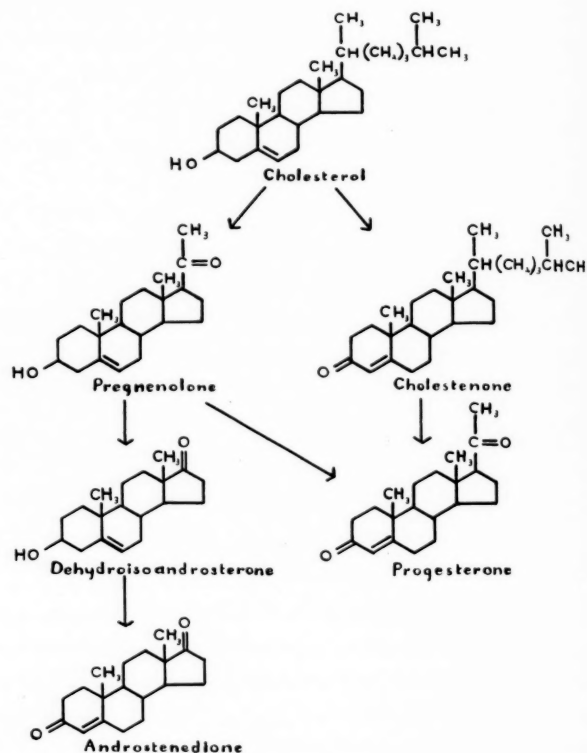


FIG. 1.—Intermediary derivatives and side reaction derivatives in the degradative oxidation of cholesterol to progesterone. In the chemical procedure the dibromide of cholesterol is used and bromine is later removed with zinc. This should be borne in mind, as it opens the possibility of reduction as well as oxidation.

SUMMARY

1. Progesterone, under the conditions that inhibited mammary cancer formation in RIII mice, failed to do so in Marsh-Buffalo mice.
2. Ovariectomized Marsh-Buffalo mice that received 10 mgm. of progesterone per mouse subcutaneously over a period of 6 months failed to develop mammary tumors.
3. Three milligrams of progesterone administered subcutaneously, alone or in combination with 500 units of estrone, failed to effect development of the mammary glands in Marsh-Buffalo female mice.
4. Mice that received sesame oil containing crude progesterone contaminated with cholestenone, or containing the equivalent of crystalline cholestenone, developed oleomas at the site of injection. Crystalline cholesterol or progesterone under the same conditions was without influence.

5. A crude synthetic progesterone made by the method of Spielman and Meyer resulted in a 32 per cent incidence of malignant tumors at the site of injection, compared with a 0 per cent incidence in controls and a 1 to 2 per cent incidence in the colony.

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Physiological Studies on Tumor-Inhibiting Agents

II. Effect on Rectal Temperatures in Normal Rabbits of the *Serratia marcescens* Tumor-Necrotizing Polysaccharide of Shear*

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INTRODUCTION

When injected into human tumor patients the highly purified, tumor-necrotizing, bacterial polysaccharide of Shear and his co-workers (9, 10, 14, 15, 16) produces a high fever (2) and other symptoms generally associated with the pyrogenic reaction (3, 4, 13). It is a matter of interest, both theoretically and practically, to ascertain what relation if any exists between the pyrogenic action of this substance and the production of hemorrhage and necrosis in certain animal and human tumors.

In the first paper of this series (1) it was reported that mice fail to show a rise in rectal temperature, no matter what amount of the polysaccharide is administered. In fact, semi-lethal and lethal amounts of the substance produce notable decreases in rectal temperature; both normal mice and tumor-bearing mice were used, and the polysaccharide was given intraperitoneally and intravenously. Thus in mice, at least, the tumor-necrotizing action of the polysaccharide cannot be attributed to a fever produced by this substance.

Pronounced elevations in rectal temperature do occur, however, in normal (*i.e.*, non-tumor-bearing) rabbits following injection of the polysaccharide, and this constitutes a major part of the subject matter of this paper. In the rabbit, at any rate, the presence of disintegrating tumor tissue is not necessary for the occurrence of the polysaccharide fever reaction.

EXPERIMENTAL

On an experimental day each rabbit was weighed, and placed for the day in an open mesh wire cage about 11 inches long, 5 inches wide, and 8 inches long, with a downswinging wire door at each end. The average rabbit sits contentedly in such a cage all day, and when removed will often jump back into it at the first available opportunity. Other advantages are that the rabbit can be kept under continual observation, and that the cage can play hardly

any role in determining the rate of heat loss. Such cages are light, cheap, easily made, and easily cleaned.

At first water was placed in the cages, but when it was found that many rabbits would go all day without drinking it was removed. This does not appear to be a cruel procedure, since at the end of day the rabbits showed no unusual eagerness to secure water, whether they had been treated with the polysaccharide or not.

Rabbits that were to receive solutions by stomach tube were deprived of food at about 5 P. M. of the day preceding the experiment.

On non-experimental days the rabbits were kept separately in cages about 2 feet square, or 5 to 15 together in a pen about 15 feet square. They were fed rabbit pellets daily and given bread, lettuce, and other green stuffs occasionally.

Copper-constantan thermocouples were used in conjunction with a Rubicon spotlight galvanometer to measure the rectal temperatures. One lead of the thermocouple was kept continuously in a water bath set at 38° C. To make a reading the back door of the small wire cage was opened, the tail of the rabbit grasped, and the second lead of the thermocouple inserted with gentle pressure at least 3 cm. but never more than 4 cm. Early attempts to make the insertion exactly 4 cm. in every case led to occasional bleeding, without any measureable change from the reading obtained when the thermocouple was inserted as far as it would go with gentle pressure beyond 3 cm. The electrical resistance was set so that 1 large scale division of the galvanometer equalled 2°C., and readings were made to the nearest 0.1°C. Readings were usually made at approximately hourly intervals, for rabbits subjected to readings at half hour intervals sometimes became difficult to handle before the end of the day. Readings were made for at least 5 hours after injection of the desired amount of the polysaccharide. Intravenous injections were made into an ear vein, with the rabbit confined in a wooden box and the head almost immobilized.

A 1 per cent solution of the polysaccharide was diluted to the desired concentration with pyrogen-free

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0.9 per cent NaCl solution, and 0.2 to 1.0 cc. of polysaccharide solution was injected for each kgm. of body weight. The 0.9 per cent NaCl solution was prepared 50 cc. at a time with C. P. NaCl and double distilled, pyrogen-free water secured from the Sharp & Dohme Pharmaceutical Company. When not in use all solutions used for intravenous injection were kept in a frigidaire. New dilutions of the polysaccharide were prepared at intervals of 2 to 4 weeks, opportunity for the formation of pyrogens in the water by bacterial contamination being thus reduced to a minimum. In 19 tests with 0.9 per cent NaCl

temperature reached, since it was found advisable not to disturb the animals by taking rectal temperatures oftener than about once an hour. The highest reading was generally obtained 3 to 4 hours after administration of the polysaccharide, and by the time 5 hours had passed the rectal temperature was usually appreciably lower than at the peak of the febrile reaction, though on very warm days the fever was prolonged.

The average rectal temperature obtained during a 5 hour period following administration of the polysaccharide was estimated from a graph of the recorded

TABLE I: EFFECT ON RECTAL TEMPERATURES IN RABBITS OF INJECTION OF VARYING AMOUNTS OF SHEAR POLYSACCHARIDE

Amt. of polysacch. injected (μ gm./kgm.)	No. of tests	H values (greatest increases within 5 hours)				A values (avg. rises during first 5 hours)			
		Highest	Lowest	Mean	S.E. of mean	Highest	Lowest	Mean	S.E. of mean
None	19 *	0.5	0.1	0.32	0.028	0.24	— 0.46	— 0.04	0.039
0.001	2	0.5	0.2			0.18	0.09		
0.005	3	1.8	0.3	1.03		1.12	0.03	0.58	
0.020	3	1.6	0.5	1.07		0.64	0.14	0.46	
0.10	4	2.4	1.1	1.88		1.51	0.73	1.19	
0.50	4	2.8	1.9	2.40		1.64	1.23	1.52	
2.0	72 **	3.2	1.3	2.34	0.017	2.31	0.87	1.63	0.010
	25 †	3.0	1.5	2.33	0.079	2.15	0.93	1.61	0.021
	4 ‡	2.5	1.0	1.53		2.15	0.70	1.20	
5.0	1 †			2.45				1.83	
10.0	2 §	2.3	2.2			1.39	1.35		
20.0	8	2.6	2.0	2.31	0.088	1.91	0.64	1.46	0.132
	3 §	1.8	1.0	1.37		1.19	0.33	0.72	
	4 ‡	2.4	1.0	1.75		1.71	0.70	1.19	
30	2 †	2.9	1.9			1.94	0.98		
40	2 †	2.8	0.8			1.82	0.56		
50	2 †	2.0	1.0 §			1.45	0.65 §		
70	2 †	2.2 §	2.1			1.66 §	1.30		
100	2 †	1.5 §	1.4 §			0.95 §	0.60 §		

* Rabbits injected intravenously with 0.2 cc. per kgm. of 0.9% NaCl; 14 of the 19 tests were made on rabbits that had never been used before.

** This column lists all data secured with only polysaccharide, in which original rectal temperature was below 41° C. (for results on 2 rabbits having higher original rectal temperatures see Table II.)

† Values obtained with rabbits that had never been injected with any amount whatsoever of polysaccharide.

‡ Values obtained after intraperitoneal injection of polysaccharide. All other polysaccharide injections into ear vein.

§ Values obtained on rabbits that died less than 24 hours after injection with polysaccharide.

solution made as described no change in rectal temperature occurred that even remotely resembled the pyrogenic reaction produced by the polysaccharide.

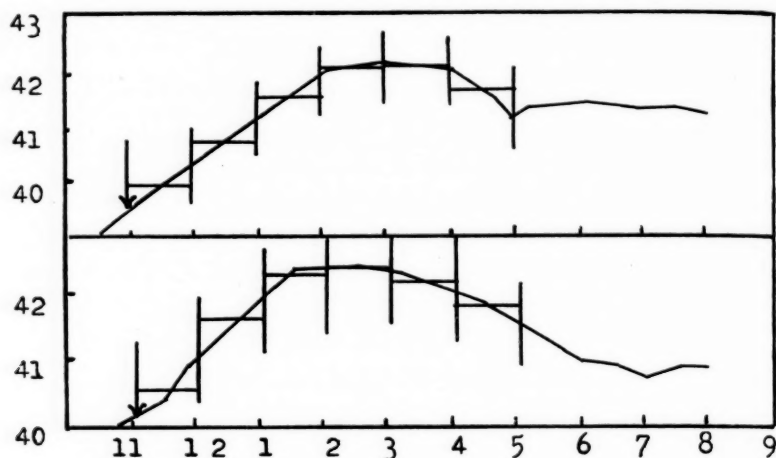
I. RECTAL TEMPERATURE CHANGES FOLLOWING INTRAVENOUS ADMINISTRATION OF VARYING AMOUNTS OF THE SHEAR POLYSACCHARIDE

The changes in rectal temperature produced by intravenous administration of varying amounts of the polysaccharide are shown in Table I. For a given test the H value was obtained by subtracting the original rectal temperature from the highest rectal temperature observed in the rabbit during a 5 hour period following administration of the polysaccharide. Usually this value was probably lower than the highest

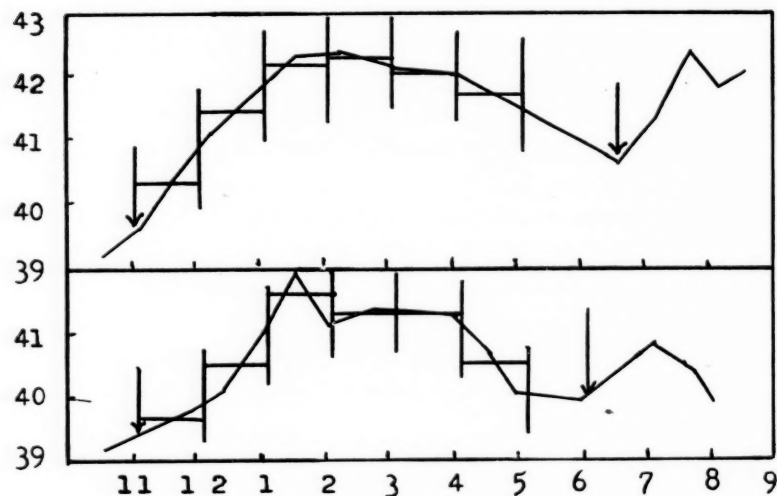
rectal temperatures (Fig. 1). The A value was obtained by subtracting the original rectal temperature from this average value, and represents, therefore, the average increase produced by the polysaccharide during the first 5 hours after its administration.

It will be noted that the average H and A values obtained for rabbits given 0.5 μ gm. of the polysaccharide per kgm. are almost the same as for those given 2.0 μ gm. per kgm., and for the 8 rabbits that survived the administration of 20 μ gm. per kgm. The response to 0.1 μ gm. per kgm. and less was smaller and much more variable. Rabbits given 20 to 100 μ gm. per kgm. were likely to show prostration, extreme muscular weakness, and more diarrhea than those receiving smaller amounts of the polysaccharide.

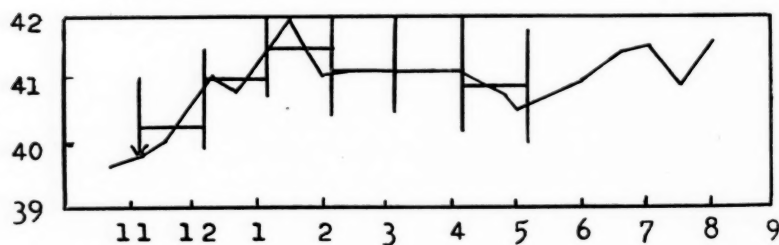
A. Rectal temperatures after single injection of 2 μ gm. per kgm.



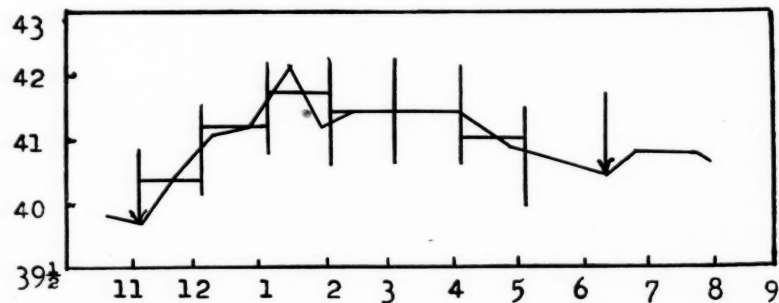
B. Rectal temperatures after 2 separate injections, each 2 μ gm. per kgm.



C. Rectal temperatures after single injection of 10 μ gm. per kgm.



D. Rectal temperatures after 2 injections, the first 10, the second 2 μ gm. per kgm.



Ordinates: ° C.
Abscissae: Time of day.
Arrows: Polysaccharide injected

FIG. 1.—Comparative effect on rectal temperatures in the rabbit of first and second injections of polysaccharide, when both injections were made on the same day.

Rabbits with extreme muscular weakness usually showed a decreased fever response also, and were quite likely to be found dead on the following morning. Two died near the end of the test in convulsions and bleeding at the mouth and nose; one had been given 50, the other 100 μ gm. of the polysaccharide per kgm. Of 7 rabbits given 20 μ gm. or more per kgm. that died less than 24 hours after the injection, 5 had a considerably decreased febrile reaction. Of 7 rabbits showing a considerably decreased febrile reaction, 5 died in less than 24 hours.

In general at least 6 days elapsed between tests made on a given rabbit. In such tests there was no evidence that previous injection with the polysaccharide had any effect on the febrile reaction. With

It is evident that the febrile reaction when the polysaccharide is administered in this manner is usually weaker and more variable than that elicited by intravenous injection.

III. EFFECT OF ENVIRONMENTAL TEMPERATURE ON ORIGINAL RECTAL TEMPERATURE AND ON THE FEBRILE RESPONSE

The present experiments were performed during the summer months in an unheated room with open windows. The average outdoor Philadelphia temperature during the experimental period of each day on which tests were made was calculated, using data secured from the U. S. Weather Bureau. The data obtained in all the tests made during the sum-

TABLE II: EFFECT OF VARIATION IN OUTDOOR TEMPERATURE ON FEBRILE REACTION AND ORIGINAL RECTAL TEMPERATURE OF RABBIT

(Polysaccharide injected intravenously, 2 μ gm. per kgm. in all tests.)

Rectal temp. at start of exper. ($^{\circ}$ C.)	Outdoor temp. below 70 $^{\circ}$ F.			Outdoor temp. 70-79 $^{\circ}$ F.			Outdoor temp. 80 $^{\circ}$ F. or more		
	No. of tests	H value	A value	No. of tests	H value	A value	No. of tests	H value	A value
38.5	1	2.9	2.06	1	1.7	0.86	0		
(38.3-38.7)									
39.0	8	2.26	1.55	7	2.61	1.84	6	2.75	1.87
(38.8-39.2)									
39.5	7	2.11	1.41	11	2.08	1.42	10	2.48	1.77
(39.3-39.7)									
40.0	2	1.95	1.42	6	2.48	1.78	7	2.34	1.79
(39.8-40.2)									
40.5	0			1	2.20	1.52	1*	2.80	—
38.3-40.7— avg. values for tests above	18	2.21 \pm 0.080	1.51 \pm 0.063	26	2.23 \pm 0.114	1.60 \pm 0.095	24	2.52 \pm 0.068	1.74 \pm 0.061
41.2				1	0.50	0.37			
41.4				1	1.20	0.22			
Average ^a values for original rectal temperatures									
	18	39.32 \pm	0.086	26	39.59 \pm	0.104	24	39.60 \pm	0.091

* Rabbit died a few minutes after rectal temperature had reached 43.5 $^{\circ}$ C. (110.3 $^{\circ}$ F.)

2 μ gm. per kgm. the average H and A values obtained in the very first tests made with 25 rabbits were identical statistically with those obtained in a total of 72 tests carried out between May and October, 1945 (Table I). In addition 5 rabbits were given the polysaccharide 4 times within 9 days; the average H and A values for the fourth time were slightly but by no means significantly lower than those for the first time.

On the other hand, 2 of 3 rabbits reinjected with the polysaccharide about 7 hours after the original injection exhibited only weak febrile reactions in response to the second injection (Fig. 1).

II. COMPARISON OF EFFECTS OF INTRAVENOUS AND INTRAPERITONEAL INJECTION OF THE POLYSACCHARIDE

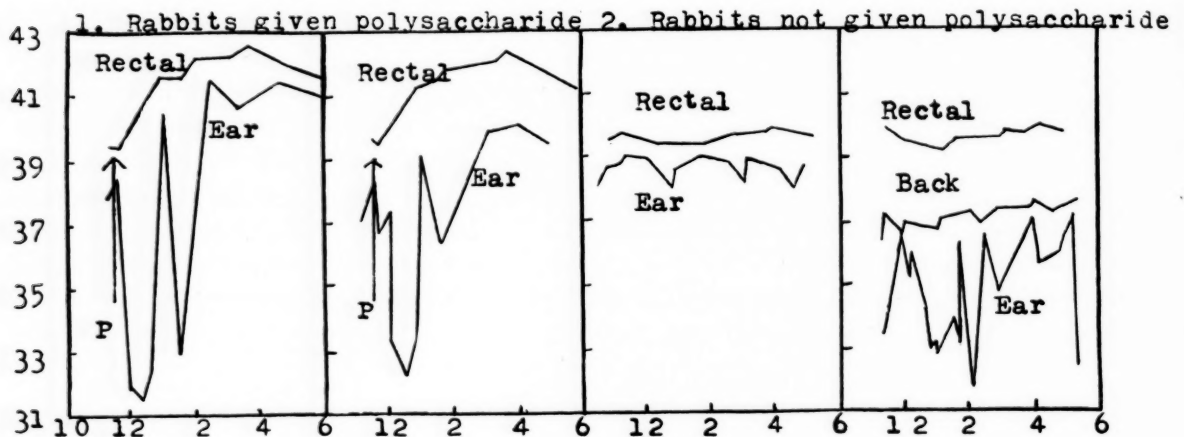
The results of the intraperitoneal injection of 2 and 20 μ gm. per kgm. also are shown in Table I.

mer months, with 2 μ gm. of the polysaccharide per kgm. of body weight, were classified according to whether the tests were made on days with average outdoor Philadelphia temperatures of (a) below 70 $^{\circ}$ F. (21 $^{\circ}$ C.): cool days; (b) 70 to 79 $^{\circ}$ F. (21 to 26 $^{\circ}$ C.): moderate days; or (c) 80 $^{\circ}$ F. (27 $^{\circ}$ C.) or more: warm days.

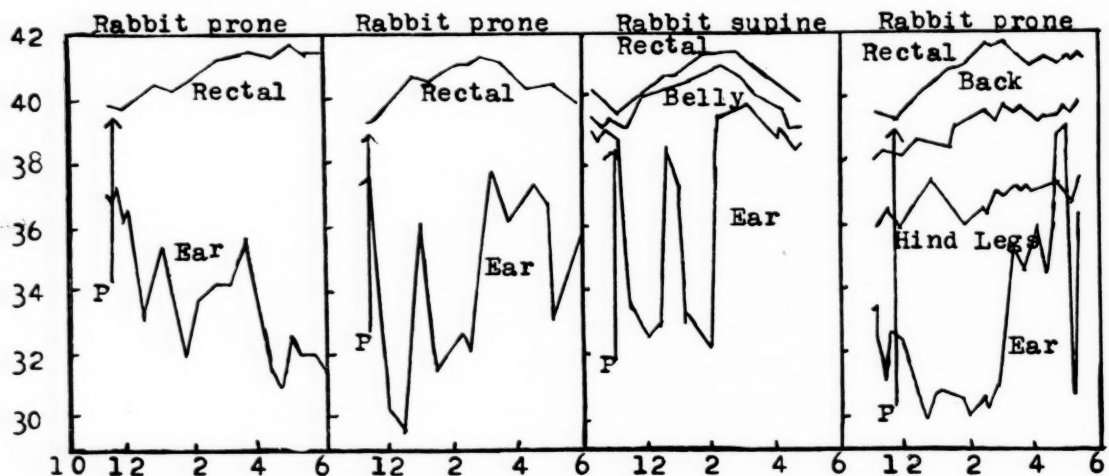
The data so obtained (Table II) indicate that on cool days the original rectal temperatures ran somewhat lower than on moderate or warm days. They indicate also that on warm days the febrile reaction was accentuated, as might be expected from the lessened heat loss on such days.

The original rectal temperature of the rabbit plays no role in determining the extent of the febrile response, unless the animal already has a fever approximating that ordinarily obtained with the polysaccharide. As shown in Table II, very small responses were observed in 2 cases in which the original rectal

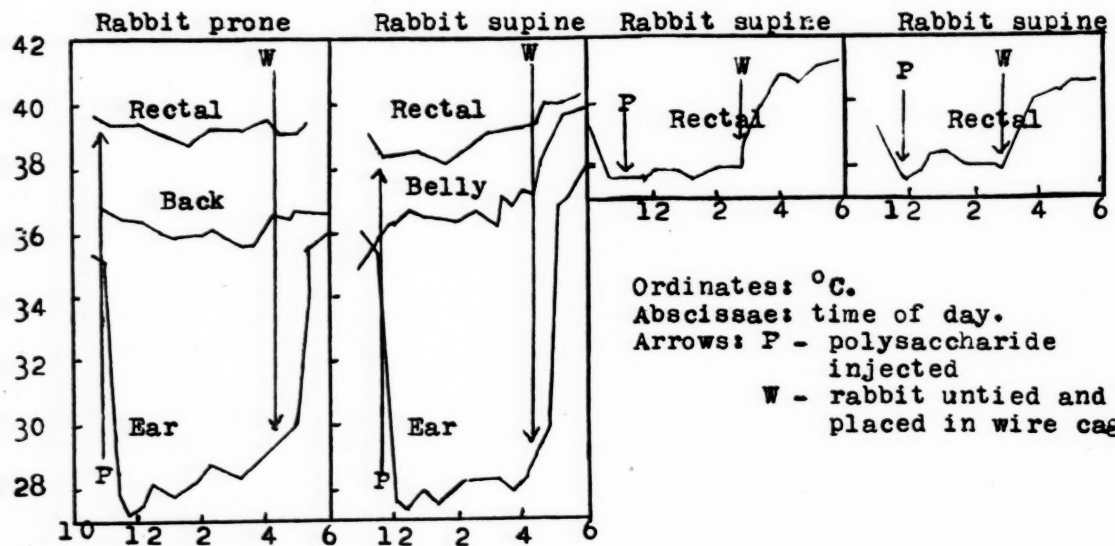
A. Rabbits sitting in wire cages



B. Rabbits tied down on cardboard and given polysaccharide



C. Rabbits tied down on copper and given polysaccharide



Ordinates: °C.
 Abscissae: time of day.
 Arrows: P - polysaccharide injected
 W - rabbit untied and placed in wire cage

FIG. 2.—Effect of contact with copper and with cardboard on polysaccharide fever.

temperatures were 41.2 and 41.4° C. On the other hand, 2 other rabbits with original rectal temperatures of 40.7° C. developed peak temperatures of 42.9° C. (109.2° F.) and 43.5° C. (110.3° F.) respectively. The first died about an hour after the peak temperature was reached, the other immediately after it had been recorded.

IV. INHIBITION OF THE FEBRILE REACTION BY PHYSICAL METHODS

A. Contact with copper sheeting.—Rabbits tied down either prone or supine in the copper trough of an unheated animal table were found not to exhibit temperature elevations after intravenous injection of 2 μ gm. per kgm. of the polysaccharide; however, in 3 of 4 tests the temperature rose to a fever level when they were untied and allowed to sit up, even when they had been tied down for as long as 7 hours after injection of the polysaccharide (Fig. 2). Rabbits tied down similarly on cardboard developed a febrile reaction after injection, which was usually somewhat smaller than that ordinarily displayed by rabbits sitting in wire cages. However, the reaction of the rabbits that were tied down was well within the range of variation in those not tied down, so that any diminution in the febrile reaction caused by the tying down is of minor importance compared with that due to enforced contact with copper sheeting.

The external ear temperatures of all rabbits dropped sharply to well below 30° C. soon after injection of the polysaccharide, whether the animals were sitting in small wire cages or were tied down on either cardboard or copper. This pronounced decrease was ordinarily followed soon by a rise, a subsequent fall, and at about the height of the febrile reaction a rise to a level higher than the original *rectal* temperature. However, rabbits tied down in the copper trough of an unheated animal table usually showed ear temperatures below 30° C. continuously for hours after injection of the polysaccharide (Fig. 2). This would suggest that heat is conducted away from its body by copper so rapidly that, in spite of the stimulus to vasoconstriction and increased heat production produced by the polysaccharide, the rabbit is unable to store up enough extra heat to raise the rectal temperature.

B. Cooling with iced water and ice (Fig. 3).—In some experiments a small wire cage containing the rabbit was half immersed in iced water before or soon after injection of the polysaccharide, to bring the rectal temperature rapidly to a low level. The cage was then put into the trough of an animal table and ice placed on the sides and tops of the cage from time to time to maintain the low rectal temperature. After some hours the ice was removed, and the rabbits were dried and placed in a dry cage. The rectal tempera-

ture then rose in each of the 6 tested beyond the original rectal temperature to a fever level.

Two rabbits treated in the same manner, except that no injection of polysaccharide was made, showed a return only to the original rectal temperature level. The rate of increase in rectal temperature was also definitely less than in the animals that had been injected with the polysaccharide.

In other experiments the rabbit was injected with polysaccharide and placed in a small wire cage; the cage was then put into the trough of an animal table and ice was piled on the sides and top of the cage in sufficient quantity to maintain the rectal temperature near its original level for 4 to 6.5 hours. In these cases, also, removal of the ice and drying of the rabbit was followed by a rise in rectal temperature to a fever level. The rise after removal of the ice was in no case so great as that which usually occurs when the fever reaction is allowed to run an unchecked course. In the few cases in which the *unchecked* fever reaction was followed for 7 to 8 hours after the injection of polysaccharide the rectal temperatures were still about as far above the original readings as was the case at any time after the removal of ice. The febrile reaction that occurred after removal of the ice was, therefore, roughly equivalent to the degree of fever that would have existed in the absence of ice, at and after the time when it was actually removed.

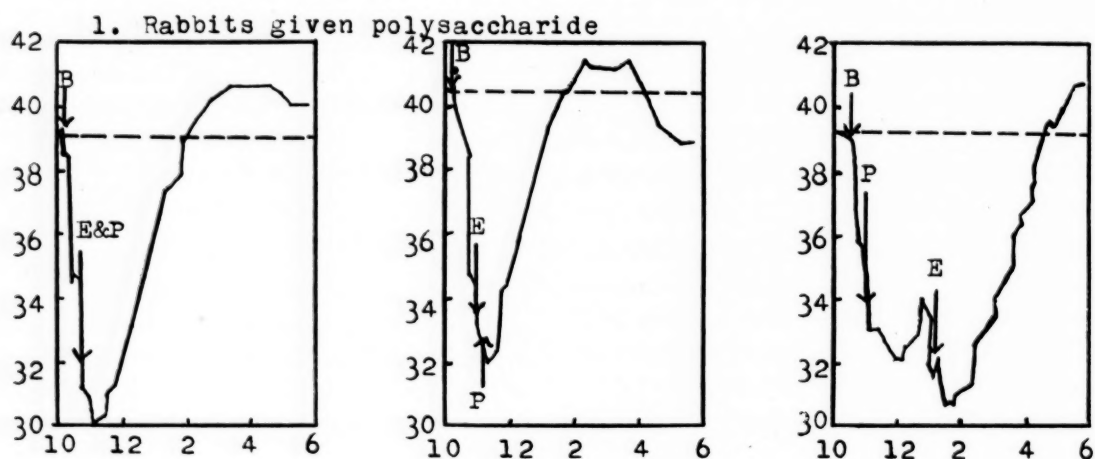
V. ACTION OF DRUGS ON THE FEBRILE REACTION

The fever produced by 2.0 μ gm. of polysaccharide per kgm. of body weight has been taken as a control value against which the antipyretic effect of a drug may be measured. Three sets of control values are available: (a) the average H and A values obtained for 72 separate determinations made during the summer months (Table I); (b) control determinations made on some of or all these rabbits at some time before and/or after administration of the drug; (c) control determinations made the same day on other rabbits. For the sake of brevity the control values described under (b) and (c) have been averaged (last 3 columns of Table III).

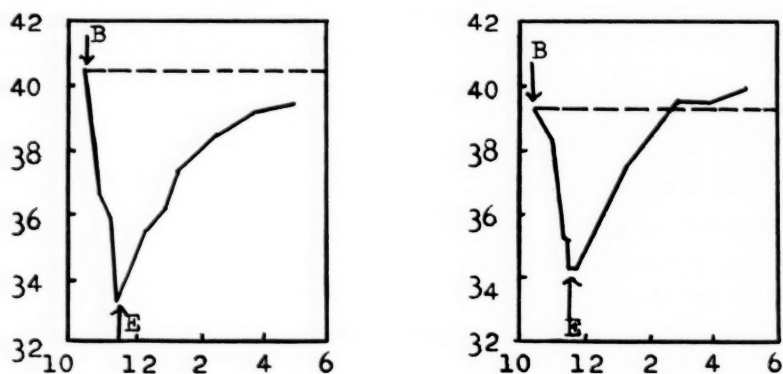
From Table III it is seen that aspirin, antipyrine, larodin (7), and dial appreciably minimized the polysaccharide fever when used in amounts that were not acutely toxic. The report by Hambourger (8) that glucono-*p*-phenetidin is an effective antipyretic has not been confirmed in the present studies. The data in the table indicate that much smaller amounts of larodin (isopropyl antipyrine) than of antipyrine are required to counteract effectively the polysaccharide fever.

However, a recasting of the data in the form of a graph of the entire febrile reaction (Fig. 4) indicates that both antipyrine and aspirin exert a much longer

A. Rectal temperatures brought to far below normal values



2. Rabbits not given polysaccharide



B. Rectal Temperatures kept close to the original level for some hours after giving polysaccharide

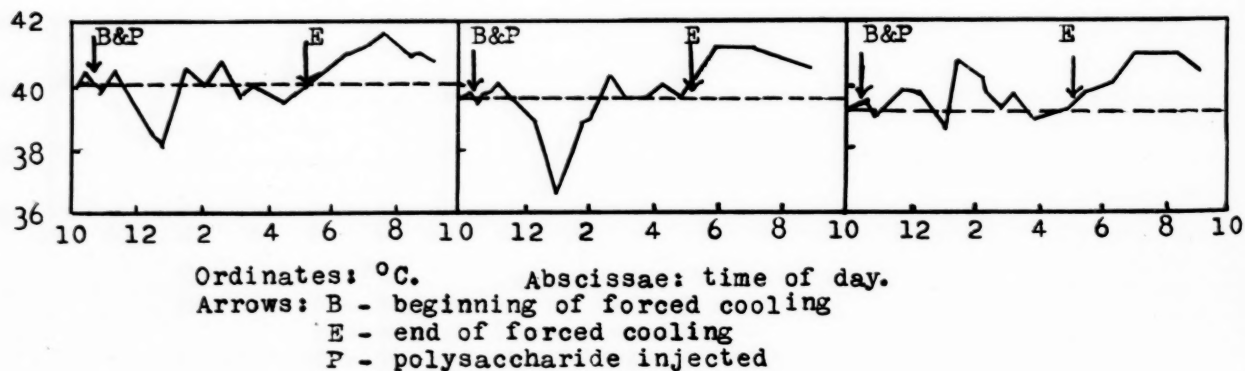


FIG. 3.—Temporary prevention of polysaccharide fever by cooling with ice water and cold air.

TABLE III: ACTION OF DRUGS ON POLYSACCHARIDE FEVER

Drug used	Amount (mgm./kgm.)	How given *	Minutes before poly.	Drug test values			Control values		
				No. of tests	Avg. H value	Avg. A value	No. of tests	Avg. H value	Avg. A value
ANTIPYRETICS									
Antipyrene	50	Vein	2	2	2.60	1.81	4	2.40	1.70
	150	Subc.	2	3	1.57	0.83	2	2.35	1.67
	100	Stom.	45	3	1.87	1.01	7	2.34	1.87
	300	"	45	10	1.07 \pm 0.062	0.64 \pm 0.057	11	2.15 \pm 0.14	1.34 \pm 0.13
Isopropyl antipyrene (larodin)	50	"	45	3	1.80	1.04	8	2.28 \pm	1.66 \pm
	100	"	30 **	9	1.30 \pm 0.20	0.71 \pm 0.15	"	0.21	0.16
Acetylsali- cylic acid (aspirin)	200	"	40	3	2.03	1.22	3	1.83	1.26
	500	"	60	3	0.23	— 0.10	5	2.20	1.58
Phenacetin	200	"	60	3	2.23	1.58	2	2.60	2.03
	500	"	60	3	1.73	1.21	"	"	"
Glucono- <i>p</i> - phenetidin	1000	"	60	7	2.05	1.35	14	2.26	1.63
	5000	"	60	1	1.60	1.02	"	"	"
HYPNOTICS AND BARBITURATES									
Chloral hydrate	300	"	45	5	2.04 \pm 0.19	1.04 \pm 0.43	7	2.44	1.81
Paraldehyde	1000	"	25	3	2.67	1.97	5	2.82	2.10
Nembutal	50	Vein	2	2	3.15	2.36	7	2.67	1.97
Na amytal	48	"	2	3	1.93	0.80	6	2.52	1.87
	60	"	2	2	1.50	0.26	4	2.20	1.50
	80-	"	Repeat † doses	6	1.93	1.23	9	2.61	1.92
	110								
Dial	20	"	2	5	2.46	1.68	8	2.45	1.78
	40	"	2	7	0.89 \pm 0.26	0.14 \pm 0.17	6	2.15 \pm 0.21	1.45 \pm 0.18
Na barbital	100	Subc.	2	3	2.20	1.55	2	2.35	1.67
OPIUM DERIVATIVES									
Morphine sulf.	15	Vein	20	5	2.36	1.50	5	2.28	1.58
Codeine sulf.	15	"	20	4	1.92	1.15	8	2.32	1.52
COMBINATIONS									
Antipyrene	300	Stom.	45	14	0.89 \pm	0.49 \pm	11	2.15 \pm	1.34 \pm
Chloral hydrate	300				0.12	0.11		0.14	0.13
Antipyrene	150	Subc.	2	3	2.07	1.44	2	2.35	1.67
Na barbital	100								

* Vein—Drug given by ear vein.

Subc.—Drug injected subcutaneously.

Stom.—Drug dissolved or suspended in 5 (or 10) per cent gum acacia and given by stomach tube, 10 cc. per kgm. (Paraldehyde given in pure form and washed down with tap water).

** Six rabbits given 100 mgm. of larodin per kgm. 30 minutes before polysaccharide. Three given 50 mgm. of larodin per kgm. 30 minutes before polysaccharide, and another 50 mgm. per kgm. 90 minutes after polysaccharide.

† Two to 3 injections per rabbit, at about 0, 2, and 3 to 4 hours after administration of polysaccharide.

effect than does larodin. This raises the question whether there is any particular advantage in administering small amounts of larodin at frequent intervals over administering large amounts of antipyrene or aspirin at longer intervals.

The graphs for dial and sodium amytal indicate that these compounds are actually less effective than the A values in Table III seem to indicate, since both produce a fall below body temperature during

the first hour after their administration. It may be noted that neither the briefly acting barbiturate, nembutal, nor the much longer acting barbiturate, sodium barbital, has any appreciable antipyretic action when used in amounts that are not acutely toxic. In fact, sodium barbital appears to counteract the antipyretic action of antipyrene, an observation of interest in connection with the report by Rentz (11) that the rectal temperature of rabbits and guinea pigs is

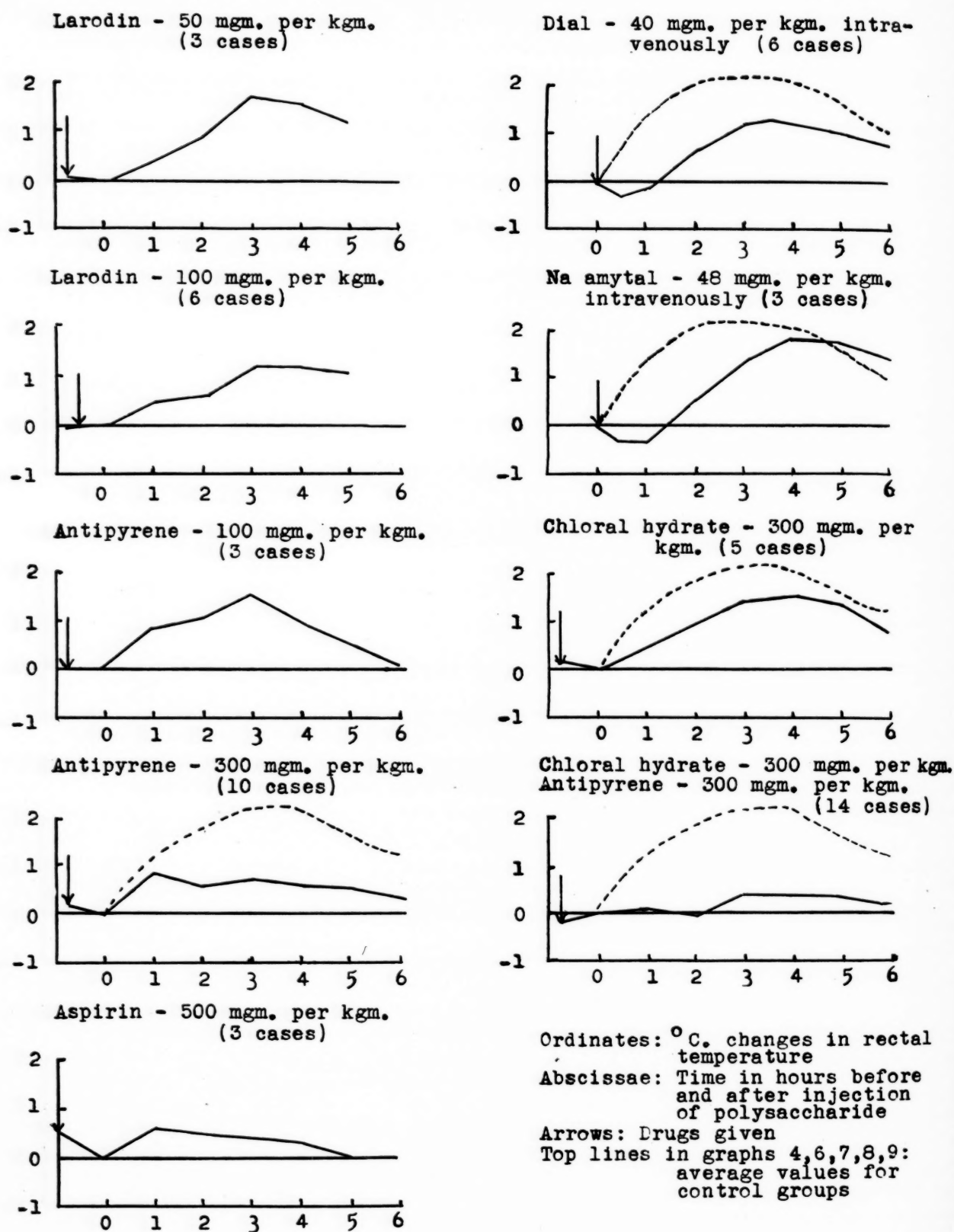


FIG. 4.—Effect of certain drugs on polysaccharide fever.

lowered from the *normal* level far more when sodium barbital is administered with antipyrene than when antipyrene is administered alone.

Rentz (12) has also reported that the rectal temperature of rabbits and guinea pigs is lowered from the *normal* level far more when chloral hydrate and antipyrene are administered together than when either one is given alone. In the present experiments chloral hydrate in the amount employed by Rentz was relatively ineffective as an antipyretic, and antipyrene was almost as effective as antipyrene plus chloral hydrate. It appears, therefore, that no predictions concerning the possible antipyretic action of drugs or combinations of drugs can be drawn from their action in lowering the body temperature from normal levels.

VI. SITE OF ACTION OF THE POLYSACCHARIDE¹

That the polysaccharide acts on the central nervous system to produce the febrile reaction rather than peripherally by stimulating cellular metabolism (as do the dinitrophenols, etc.) is indicated by: (a) the extremely minute amounts required to elicit a fever reaction (Table I); (b) the very rapid and pronounced drop in the temperature of the ear, indicating vasoconstriction, that occurs very shortly after intravenous injection of the polysaccharide; (c) the ability of certain antipyretics to counteract almost completely the fever (fevers produced by substances that stimulate cellular metabolism are relatively refractory to antipyretic drugs).

We secured additional evidence for central action of the polysaccharide by cutting the spinal cord at the height of the fever (rectal temperature, 41.8° C.). The temperature started to fall immediately, precipitously (over 2° C. per hour), and at a remarkably constant rate, reaching 38.6° C. in 92 minutes, at which time the animal stopped breathing. The rectal temperature in another rabbit in which fever had been induced by the injection of dinitrophenol (15 mgm. per kgm.) rose 0.5° C. further during 13 minutes after the cord was cut. At this time the rectal temperature had reached 42.4° C. and the rabbit stopped breathing.

In both experiments the spinal cord was exposed under ether anesthesia and a thread placed under it. After the rabbit had regained consciousness and the rectal temperature had returned close to the original level the fever-producing substance was injected intravenously (2 µgm. of polysaccharide per kgm. in the one case, 15 mgm. of dinitrophenol per kgm. in the other) and the rectal temperature measured at about 20 minute intervals until the desired fever level was reached. At this time novocaine was injected into the cord and the cord cut. Since the cord was already

exposed the injection and the cutting required only a few seconds.

In still a third experiment a rabbit was held under ether anesthesia for the length of time required in the other 2 experiments to expose the spinal cord. Polysaccharide was injected after recovery of consciousness and the original rectal temperature. In this experiment the height of the fever reaction (42.2° C.) was reached 156 minutes after injection of the polysaccharide; 204 minutes later the rectal temperature had fallen to 40.4° C., the average rate of fall being 0.53° C. per hour, or about ¼ as rapid as that exhibited by the rabbit in which the cord had been cut.

DISCUSSION

Co Tui and his co-workers (4) have defined as a minimum pyrogenic dose (MPD) the smallest amount of pyrogen that on intravenous injection provokes a rise in body temperature of 0.5 to 0.6° C. within 4 hours after injection. They report for a polysaccharide from *Eberthella typhosa* purified by them an MPD of 0.06 µgm. per kgm. for the rabbit. Robinson and Flusser (13) have given data on temperature increases in the rabbit produced by 3 polysaccharides isolated and purified by them from triple vaccine (*Eberthella typhosa* plus *Bacillus paratyphosa* A and B), *Pseudomonas aeruginosa* (*B. pyocyaneus*) and *Proteus vulgaris*. Their data suggest that the MPD's for the pyrogens they purified would in no case be appreciably less than 0.1 µgm. per kgm. The data in Table I of our paper indicate that the MPD for the tumor necrotizing polysaccharide of Shear employed in the present experiments is between 0.001 and 0.005 µgm. per kgm. for the rabbit.

Maximum increases of the order of 2 to 3° C. were regularly obtained with the Shear polysaccharide in amounts as low as 0.5 µgm. per kgm. The data of Robinson and Flusser (13) indicate that they required at least 25 µgm. per kgm. of the triple vaccine polysaccharide to secure comparable maximum increases. In no paper dealing with experiments on rabbits that have come to our attention have increases as great as 3.0 to 3.3° C. been reported, though such were obtained in several instances in the present experiments. We conclude, therefore, that the Shear polysaccharide is the most potent highly purified pyrogen of polysaccharide structure available at the present time.

The increases in rectal temperature produced in the dog and guinea pig by the Shear polysaccharide in the experiments of Franke (5) were smaller and more variable than those reported here for the rabbit. Many of Franke's experiments were carried out under anesthesia. More important, in the experiments for which he has reported rectal temperature values, he used only semi-lethal amounts of the polysaccharide, the smallest apparently being 1,000 µgm. per kgm.,

¹ With the assistance of Dr. M. J. Dresbach, of the Department of Physiology, Hahnemann Medical College.

whereas in the present experiments the largest amount of the polysaccharide employed was 100 μ gm. per kgm. In these, 5 out of 7 rabbits that died in less than 24 hours after having been injected with somewhere between 20 and 100 μ gm. of polysaccharide per kgm. showed very weak febrile reactions, and only 1 of the 7 had a reaction in the range typical for rabbits receiving 0.5 and 2.0 μ gm. per kgm. There is a good possibility, therefore, that smaller amounts of the polysaccharide might produce more pronounced febrile reactions in the dog and the guinea pig.

It is interesting to note that, per unit of body weight, the rabbit is killed by much smaller amounts of the polysaccharide than is the guinea pig, the dog, or the mouse. The data in Table I indicate that the half lethal dose for the rabbit is greater than 20 and is probably not greater than 100 μ gm. per kgm. Franke (5, 6) estimated the half lethal dose for the guinea pig as about 1,000 to 2,200 μ gm. per kgm., and stated that his data indicated a half lethal dose for the dog of about the same order of magnitude. The half lethal dose for the normal mouse is about 100 μ gm. per mouse, and for a mouse bearing primary subcutaneous tumors about 15 μ gm. per mouse. For a body weight of 25 gm. these values are equivalent to 4,000 and 670 μ gm. per kgm. of body weight.

CONCLUSION

1. The tumor-necrotizing polysaccharide employed in the present experiments produced measurable increases in rectal temperature in the rabbit when given intravenously in amounts as low as 0.005 μ gm. per kgm.

2. An elevation of from 2 to 3° C. was usually produced when the polysaccharide was given intravenously in amounts equal to or greater than 0.5 μ gm. per kgm. This elevation was generally accompanied by diarrhea.

3. Death occurred in some of the rabbits given 20 to 100 μ gm. of polysaccharide per kgm. The rabbits that died usually had a rather weak febrile reaction and greatly diminished muscular strength. In all cases death occurred in less than 24 hours after the injection. The 2 rabbits that died during the experiment went into convulsions, and 1 bled at the nose and mouth.

4. The febrile reaction was intensified and prolonged on very warm days.

5. Elevation of the rectal temperature did not occur as long as the rabbit was tied down on a copper table, or cooled with iced water and cold air.

6. It was possible to minimize the febrile reaction with the following drugs, in amounts that were not acutely toxic to the rabbit: antipyrine, isopropyl antipyrine (larodin), acetyl salicylic acid (aspirin), and dial. A number of other drugs, when used in the amount and manner specified, were ineffective.

ACKNOWLEDGMENTS

We are indebted to Dr. M. J. Shear, of the National Cancer Institute, Bethesda, Md., for much valuable advice and the sample of polysaccharide employed; to Chas. Pfizer Co., New York, N. Y. for the glucono-*p*-phenetidin; to Hoffman LaRoche Co., Nutley, N. J., for the isopropyl antipyrine (larodin); to Dr. R. Beutner, Department of Pharmacology, Hahnemann Medical College, for most of the other drugs used; and to Mr. E. Mendelson, Department of Physiology, University of Pennsylvania, for preparation and repair of the thermocouples employed.

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Induction of Sarcoma of the Liver in the Rat with Methylcholanthrene and Benzpyrene

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In studying the effects of the carcinogenic hydrocarbons these substances are most commonly applied to the skin or injected subcutaneously for obvious reasons. No doubt there are variations in the sensitivity of different animals, as well as of the different tissues of individual animals; as examples may be cited the extreme reactivity of mouse skin and of mouse and rat subcutis, which are in sharp contrast to the high resistance characteristic of rat skin. Nevertheless, as proof of the general effectiveness of these agents in the production of neoplasia, the literature abounds in examples of tumors induced in practically all tissues. But the ability of the carcinogenic hydrocarbons to produce new growths in the liver has been seriously questioned by a number of investigators, who found minimal or nonspecific reactions in this organ after exposure for considerable periods (5, 8, 9, 12). Shear, Stewart, and Seligman (9) state that this apparent resistance of the liver does not depend upon absorption or removal of the chemicals, for pellets that had lain in the mouse liver for as long as 16 months without inducing malignant disease were found intact, and surrounded by only a mild tissue reaction. Still, their protocols include a description of an adenocarcinoma that was found in the liver of a mouse of the C57 black strain, in which spontaneous hepatic neoplasms do not occur, 12 months after the insertion of a thread coated with dibenzanthracene; the authors favor an origin of this induced tumor in the biliary ducts or gall bladder because of its histology and location.

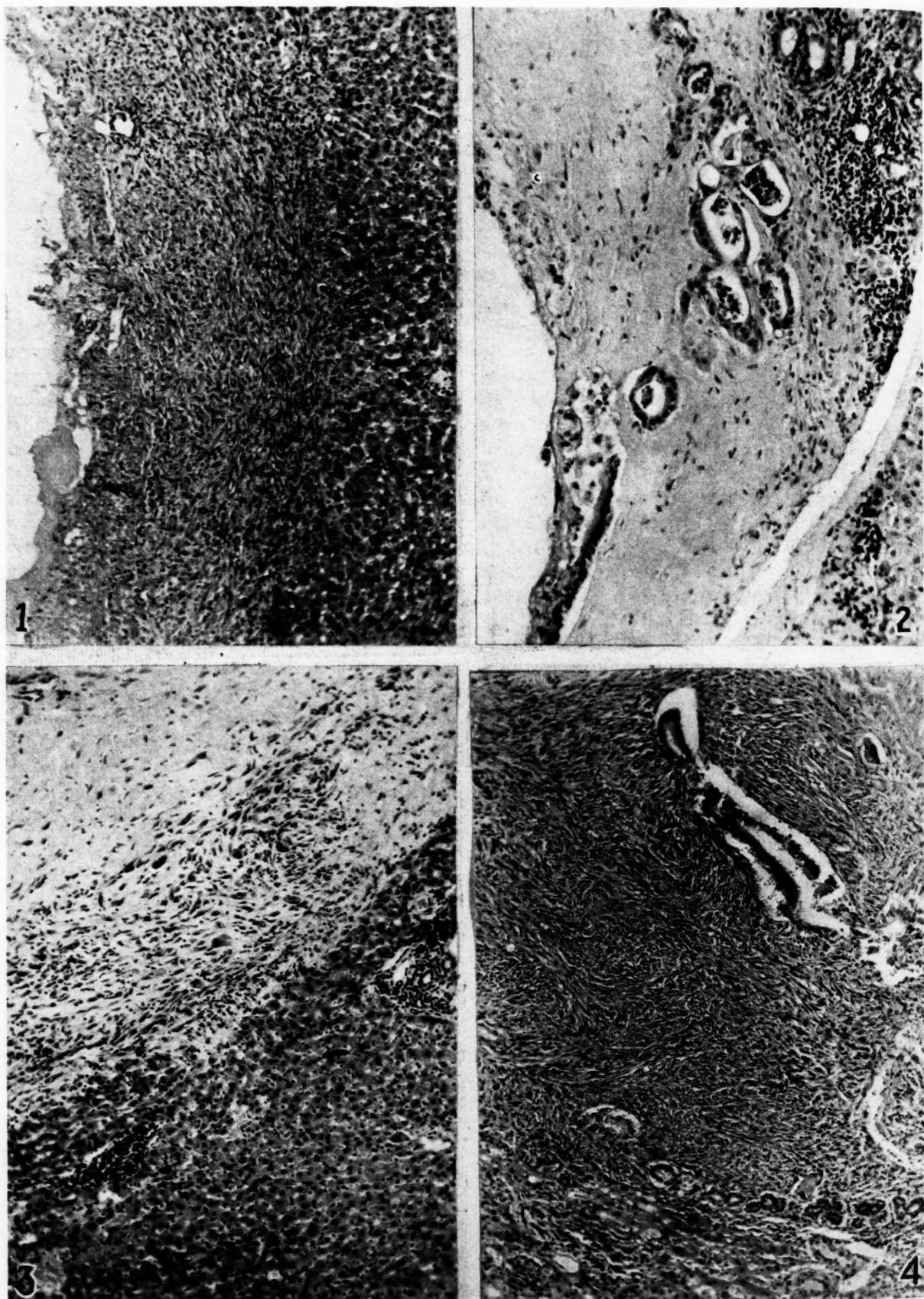
Oberling and the Guérins (7), on the other hand, recorded 2 examples of sarcoma of the rat liver in animals examined 16 and 25 months after an implant of benzpyrene crystals. Strong (10) succeeded in producing carcinoma of the liver in 19 of 1,367 NHO mice at an average age of 406.2 days, by injecting methylcholanthrene subcutaneously at 60 days of age; spontaneous hepatic tumors do not occur in this strain. Recently White and Eschenbrenner (11) reported the occurrence of multiple nodules, interpreted

as hepatomas, in the cirrhotic livers of 2 of 6 rats that survived 14 months on a basal diet containing 60 mgm. of 1,2-benzanthracene per 100 mgm. of food. This parent hydrocarbon possesses only negligible carcinogenicity when injected subcutaneously or painted on the skin. That the liver is endowed with no general resistance to carcinogenic agents such as might be linked with its extensive detoxifying powers is clearly evident from the ease of production of parenchymal cell neoplasia in the rat with azo compounds administered orally or parenterally, although they are ineffective when applied directly to the liver (6); or of sarcoma in this animal with *Cysticercus* larvae (1). There is much of value in the observation of Dunning, Curtis, and Bullock (2) who, on the basis of a study of subcutaneous and hepatic tumors induced by the carcinogenic hydrocarbons and *Cysticercus* disease respectively in rats, concluded that "the potency for malignancy must be a universal cell characteristic and that the histogenesis of these tumors was determined by the fortuitous exposure to the irritant of the various types of cells."

The present note records the induction of 7 hepatic sarcomas in rats bearing implants of methylcholanthrene or benzpyrene in the liver. The experiments were performed between 1941 and 1943, and were not reported earlier because of the absence of the author in military service.

MATERIALS AND METHODS

Attempts were first made to inject the carcinogenic hydrocarbons dissolved in melted paraffin (m.p., 48° C.), as had been done with ease and excellent results subcutaneously (3, 4); the liver was exposed by a midline upper laparotomy. This method did not prove feasible, however, because of the technical difficulties produced by the small anteroposterior dimension of the organ, its mobility, and its friability. In almost all of several dozen attempts the material either solidified in the syringe before the injection could be started, or if sufficient speed had been at-



FIGS. 1-4

tained to avoid this it was found subsequently to have entered the spaces between the hepatic lobes.

Insertion of a solid pellet of paraffin, 0.25 cc. in volume and containing 1 mgm. of methylcholanthrene or benzpyrene, in the exposed median or left lateral lobes proved a simple and adequate method of administration. Benzpyrene is readily soluble in paraffin in the 4 per cent concentration necessary to give the dose mentioned above, but with methylcholanthrene a small persistent sediment of insoluble material remained despite prolonged heating on a water bath at boiling temperature. By agitating these crystals before solidification of the paraffin, however, relative uniformity in dosage could be achieved. To make the pellets the melted material was drawn into a 5 cc. pipette of which the mouthpiece and tip had been cut off, leaving a cylindrical tube. Oil of sesame had been previously drawn into and drained from the pipette to coat it with oil and insure facility of expulsion of the solidified paraffin by gentle pressure at either end. Quantities of 0.25 cc. were expelled, severed with a sharp blade, and the resultant flat discs fashioned into oval pellets by rolling them between gloved fingers. To insert a pellet in the liver a small incision was first made in the anterior aspect of the capsule, and the mass inserted into the yielding tissue with slight pressure of a small forceps. If the pellet was placed deeply enough it remained *in situ* without suture of the capsule.

A total of 51 rats were thus tested for the effect of carcinogenic compounds on the liver: 25 with methylcholanthrene and 26 with benzpyrene. As controls, 14 animals received these pellets subcutaneously in the right flank, and 15 other rats received pellets of paraffin alone in the liver.

In addition, cotton threads saturated with methylcholanthrene while this was in the molten state were sutured in the livers of 4 animals. The dose of carcinogen was calculated by weighing measured portions of the thread before and after impregnation. The prepared thread was pulled through the liver with an ordinary straight needle, and severed flush with the capsule at its points of entrance and exit. Approximately 1 cm., which contained 0.8 mgm. of the compound, was employed in each animal.

The rats, males and females of the August, Sherman, and Wistar stocks, varied in age from 91 to 161 days at the outset of the experiment, with the exception of one group of 10 older animals of 273 to 296 days. Their diet consisted of a mixture of Purina and Rockland chows, adequate water, and a generous portion of fresh carrots once weekly, and their nutritional status continued good, excluding those animals that developed the cachexia of late malignant neoplastic disease.

RESULTS

Three well defined stages of reaction occurred about the pellets containing methylcholanthrene or benzpyrene: acute inflammation, healing with fibrosis, and tumor formation.

1. *Inflammatory reaction.*—Pellets of animals that died during the first 3 weeks after implantation of a carcinogen were surrounded by an acute, nonspecific, inflammatory response (Fig. 1). The exudate consisted of fibrin, polymorphonuclear leukocytes, and lymphocytes and was accompanied by many newly formed small blood vessels. A considerable number of fibroblasts appeared early. The reaction was without doubt dependent upon a combined response to the insults of mechanical trauma and a foreign body, and did not differ from that produced by control pellets of paraffin alone. The hepatic cells beyond the immediate area of reaction remained unaltered, but a few, in proximity to the pellet, exhibited such signs of disintegration as shrunken nuclei, fatty cytoplasm, or frayed outlines.

2. *Fibrosis of capsule of pellet.*—With subsidence of the active inflammatory response after 2 or 3 weeks progressive fibrosis and hyalinization developed (Fig. 2). Pellets examined then, and at intervals up to 90 weeks as animals died of intercurrent disease, were surrounded by mature connective tissue fibers (unless neoplastic transformation had supervened). With increasing age of the pellet these fibers became progressively more acellular and appeared more dormant and better demarcated from the adjacent unchanged liver. Scattered lymphocytes often persisted in the sclerotic bundles, and the bile ducts ensnared in this fibrous tissue were often distorted, enlarged, or ab-

DESCRIPTION OF FIGURES 1 TO 4

Fig. 1.—Acute inflammatory reaction 10 days after insertion in liver of paraffin pellet containing 1 mgm. benzpyrene. Cavity to left represents pellet dissolved during preparation of histologic section. August male. Mag. $\times 100$.

Fig. 2.—Fibrotic replacement of active inflammation 76 days after implant of 1 mgm. benzpyrene. Persisting distorted bile ducts and foci of lymphocytes can be observed. Sherman female. Mag. $\times 135$.

Fig. 3.—Early sarcoma of liver in periphery of fibrotic tissue

about pellet, 248 days after implant of 1 mgm. benzpyrene in paraffin. Hyperchromatic cells of bizarre shape, and mitotic figures, are present. Fibrotic acellular areas in upper left are a portion of reaction about pellet. Sherman female. Mag. $\times 100$.

Fig. 4.—Spindle cell sarcoma of liver 485 days after implant of 1 mgm. methylcholanthrene in paraffin. Tumor contains remnants of distorted bile ducts. Fibrotic zone in upper right is a portion of inner, uninvolved zone of reaction about pellet. Sherman male. Mag. $\times 100$.

normally grouped in small clusters. At times their individual cells, with their abnormally eosinophilic cytoplasm, were larger and more conspicuous than normal, but never gave any indication of neoplastic change. The morphology of this scarred reaction tissue about the pellets that contained the carcinogens differed in no detail from that surrounding the control pellets of paraffin.

A study of serial sections of the tissue around a considerable number of carcinogenic pellets, at various stages when no gross tumor or microscopic evidence of neoplasm was found in a single section, gave no clue to the changes that precede neoplastic transformation. A tumor, when present, exhibited the characteristic histologic criteria, and little could be learned of the important preliminary stage.

3. *Tumor formation.*—Sarcoma of the liver in the area immediately about the implanted carcinogen occurred in 7 of 27 rats that survived 248 days, the minimum period for initiation of the malignant process. Evidence of a tumor at this time was found only after the examination of serial sections (Fig. 3). The difference between this tissue and the benign reaction is striking. The relative cellularity and the presence of many hyperchromatic cells of bizarre shape, together with mitotic figures, among the bands of more delicate spindle elements, permitted a diagnosis of early sarcoma, especially as tumors in other animals consisted merely of larger accumulations of these altered cells. It is interesting to note that the earliest sarcoma in the series occurred at the periphery of the fibrous tissue about the pellet; at some distance, therefore, from the area of direct contact with the carcinogen, as others have observed, rather than at its point of strongest concentration.

Another tumor (Fig. 4) was a well developed but small spindle cell sarcoma, found in an animal dead 485 days after implantation of a methylcholanthrene pellet. This neoplasm, too, appeared to offer evidence of the initiation of the malignant alteration at the periphery of the reaction tissue, for it invaded hepatic parenchyma, engulfed a number of enlarged, distorted bile ducts, and encroached upon the hyalinized, acellular tissue immediately about the carcinogen pellet; but the persistence of a rim of benign fibrous tissue in the area closest to the carcinogen indicated that the neoplasm arose exterior to this site.

The remaining neoplasms arose in an animal dead 411 days after receiving benzpyrene, and in 4 others 389, 449, 510, and 630 days following the insertion of methylcholanthrene; all were larger growths, attaining a maximal size of 5 cm. in their largest dimension. Two of the methylcholanthrene tumors were in animals that had received the chemical in threads. In all cases the responsible agent was found

embedded in the neoplasm, whose large size precluded discovery of the site of origin. Liver tissue at a distance, when not involved by the expanding tumor, remained unaltered both in the gross and microscopically. Widespread peritoneal implantation metastases from the pelvis to the under surface of the diaphragm were found in one animal of this group. The tumors proved to be spindle cell sarcomas with zones of a more polymorphous architecture distributed throughout at random. Neoplastic alteration of the hepatic parenchyma was not encountered. Inadequate specific irritation of its cells by the carcinogen might account for this; either because of their relative distance from the agent, separated as this was by the fibrous tissue about the pellets, or because a sarcoma destroyed the animal before a tumor could develop from the epithelium. On the other hand, insusceptibility of the epithelium to carcinogenic hydrocarbons may be an underlying factor.

With the exception of the opportunity of estimating the latent period for benzpyrene, in the 1 animal that died early in the course of sarcoma development, the time of onset of the tumors could not be determined. The other growths had obviously been present for a time when the animals died; in some cases slowly growing masses had been detected for some weeks prior to death, but palpation of a deeply situated internal neoplasm that does not grow rapidly cannot give even approximate information on its time of onset. Of the 15 rats receiving control implants of paraffin alone in the liver, 8 survived for periods of 505 to 553 days. The reaction about the pellets in these animals consisted of dense hyalinized connective tissue, with no evidence of neoplastic transformation.

Subcutaneous tumors produced by methylcholanthrene and benzpyrene pellets.—Pellets identical with those used in the liver experiment were implanted into 14 rats subcutaneously in the right flank, through a small skin incision. Tumors developed in 10 animals, and their relatively slow growth paralleled in most instances that of the hepatic neoplasms. The presence of a tumor was determined by the first palpable enlargement of the implanted pellets, and on this basis the minimal latent period was 119 days. No estimate can be given of the time of development of microscopic tumors. Tumor rats survived for 225 days to 528 days before the neoplasms had attained sufficient size, from 5 to 9 cm. in greatest dimension, to make it expedient to sacrifice the animals. All subcutaneous tumors were spindle cell or polymorphous cell sarcomas. The shorter latent period and greater yield of tumors in the subcutis as compared with the liver suggest speculation on the lesser sensitivity of the latter to the carcinogenic hydrocarbons;

however, the number of animals employed in this experiment was too small to warrant any extensive elaboration of such a hypothesis. Full consideration of differences in susceptibility of various tissues to the carcinogens has been given by Shear, Stewart, and Seligman (9).

SUMMARY

Sarcoma of the liver in the rat was produced in 7 of 27 animals that survived 248 days, the time required for the induction of the earliest tumor, after intra-hepatic implantation of paraffin pellets containing 1 mgm. of methylcholanthrene or benzpyrene, or of cotton threads impregnated with 0.8 mgm. methylcholanthrene.

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Nucleic Acids in Human Tumors*†

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Nucleic acids and nucleoproteins are important constituents of the cell, with vital roles to play in the transfer of genic characteristics, in cellular division, in the synthesis of proteins, and probably in the formation or action of enzymes (6, 11, 14, 26). The increasing evidence that disturbances in the nucleoproteins and nucleic acids may be an important factor in the intracellular cause of tumors has been recently reviewed (26). Photometric histochemical observations have shown that there are increased amounts of thymonucleic acid in some epidermoid carcinomas of the skin of men (29), and in the epidermis of mice painted with methylcholanthrene (22) as well as in the white blood cells of patients with lymphoid leukemia (25).

If quantitative differences in nucleic acids represent an intracellular cause of neoplasia, it should be possible to demonstrate these changes in a significant percentage of many different types of new growth. The following photometric histochemical study of 20 human tumors was undertaken to determine the variations in nucleic acids of the desoxyribose and ribose types in a variety of neoplasms and corresponding normal tissues. For the proper interpretation of quantitative chemical measurements it is desirable to relate the results to the living biologic unit, the cell, and to variations in its morphologic constituents. Therefore the results of these measurements are related as far as possible to the volume of the tissue and to the mean volume of the cell, nucleus, cytoplasm, chromatin, nucleolus, and nuclear sap.

MATERIALS AND METHODS

The 20 specimens for analysis were chosen from a large number of surgical and autopsy specimens because they showed negligible artifacts and represented

a variety of tumors suitable for analysis. Since measurements on neoplastic and corresponding homologous normal tissue were to be compared, specimens were selected in which the normal and malignant tissues were in close proximity so that they could be prepared for analysis as a single block of tissue and

TABLE I: SPECIMENS FOR ANALYSIS OF NUCLEIC ACIDS

Specimen no.*	Fixation of tissue	Diagnosis
11336	Zenker-formol	Primary carcinoma, liver
11120	Formaldehyde	Leiomyosarcoma, portal vein
6989	Zenker-formol	Epidermoid carcinoma, epiglottis, Gr. II
7362	"	Epidermoid carcinoma, larynx, Gr. III
7306	"	Epidermoid carcinoma, larynx, Gr. III
6894	Zenker-acetic	Epidermoid carcinoma, larynx, Gr. II
1582	Sublimate-alcohol	Epidermoid carcinoma, face, Gr. II
5631	Formaldehyde	Transitional cell carcinoma, nasopharynx
10827	"	Transitional cell carcinoma, urinary bladder
43-939	Sublimate-alcohol	Basal cell carcinoma
45-328	"	Turban tumor, scalp
11508	Zenker-formol	Renal cell carcinoma
11364	Formaldehyde	Adenocarcinoma, prostate
11338	"	" stomach
11077	Zenker-formol	" colon
11384	Formaldehyde	" "
45-48	Sublimate-alcohol	" rectum
45-142	"	" "
11223	Zenker-formol	" bronchus
11375	Formaldehyde	" "

* Specimens numbered above 10,000 were from the autopsy material of the Department of Pathology; below 8,000 were surgical specimens from the Department of Otolaryngology of Washington University School of Medicine. The 3 preceded by 45- were from the surgical material of the Ellis Fischel State Cancer Hospital, and 43-939 was from The Barnard Free Skin and Cancer Hospital; Dr. Lauren V. Ackerman and Dr. Zola K. Cooper cooperated by furnishing these 4 specimens for analysis.

measured in the same microscopic section. The sources of the 20 tissues, the fixation, and the pathologist's diagnosis are shown in Table I. Specimens obtained more than 2 hours post mortem were not used.

The relative content of desoxyribose nucleic acid was measured indirectly by staining with the Feulgen reaction for thymonucleic acid and measuring the absorption of light from the stain within the nuclei.

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The details of the method have already been described (22, 23).

The photometric instrument for the measurement of pigment or stain in smears or sections of tissue consists of a light source with filters, a microscope, photocell, and amplification and recording apparatus (22). The sections are prepared and stained with meticulous care to give uniform results, and adequate precautions are taken to correct for variations in

of cells, including blood and inflammatory cells; (c) necrosis and (d) tissue spaces, may be recognized and deleted from the measurements. In this way a small sample of tissue that is not diluted with extraneous material can be obtained for analysis more readily than with most macrochemical methods. The advantages of objective, accurate measurements with a sensitive photocell over less accurate opinions gained through estimation by the eye are obvious.

TABLE II: RELATIVE THYMONUCLEIC ACID CONTENT PER UNIT VOLUME AND PER CELL OF NORMAL AND NEOPLASTIC TISSUES, AS MEASURED BY LIGHT ABSORPTION OF TISSUE STAINED BY FEULGEN REACTION

Specimen no.	Tissue	Size of tissue volumes measured	Light absorption measurements							
			Per volume				Per cell			
			% Mean absorption	Coeff. of variation %	Ratio of absorption T/N	P	% Mean absorption	Coeff. of variation %	Ratio of absorption T/N	P
11336	Liver	Large	11.2	12.5	1.98	.0000	1.91	22.0	1.68	.0000
	Carcinoma		22.2	17.7			3.10	21.6		
11120	Muscle	"	6.8	22.4	2.01	.0000	1.63	43.2	1.07	.3594
	Myosarcoma	"	13.7	17.8			1.74	22.2		
6989	Epidermis	"	13.4	26.0	2.18	.0000	1.70	23.0	1.08	.2296
	Carcinoma	"	29.2	16.0			1.84	17.4		
7362	Col. ep.	"	23.7	22.2	1.34	.0010	1.14	20.0	1.56	.1788
	Sq. ep.	"	14.5	38.4	2.18	.0000	1.53	30.0	1.16	.0002
	Carcinoma	"	31.7	14.0			1.78	14.5		
7306	Epidermis	"	16.0	49.3	1.31	.4364	1.41	39.8	1.19	.1357
	Carcinoma	"	21.0	20.2			1.69	20.1		
6894	Epidermis	"	14.0	27.3	1.42	.0032	1.42	28.6	1.09	.2483
	Carcinoma	"	19.9	21.5			1.55	19.3		
1582	Epidermis	"	17.1	32.2	1.47	.0022	1.40	23.8	1.50	.0000
	Carcinoma	"	25.1	25.6			2.10	15.2		
5631	Trans. ep.	"	21.0	20.4	1.26	.0125	1.58	18.8	.92	.2033
	Carcinoma	"	26.5	18.6			1.46	16.6		
10827	Trans. ep.	Small	26.5	25.6	1.16	.1469	6.95	17.8	1.90	.0018
	Carcinoma	"	31.1	28.5			13.20	37.8		
43-939	Basal ep.	"	25.2	28.8	1.34	.0233	7.00	22.3	.95	.3409
	Carcinoma	"	33.8	26.8			6.66	21.9		
45-328	Basal ep.	"	23.2	19.5	1.56	.0000	5.73	23.8	1.29	.0139
	Sweat gl.	"	33.2	17.7	1.09	.1685	6.93	18.3	1.06	.0287
	Carcinoma	"	36.3	15.2			7.38	19.8		
11508	Renal tubules	"	18.4	21.1	1.16	.0630	6.97	23.6	1.10	.1685
	Carcinoma	"	21.3	16.6			7.68	17.6		
11364	Prostate gl.	"	31.2	24.0	.78	-.0179	6.25	22.1	1.58	.3015
	Adenoca.	"	24.2	22.6			6.62	19.4		
11338	Gastric gl.	"	17.6	34.2	2.09	.0000	6.48	34.1	1.82	.0004
	Adenoca.	"	36.8	26.7			11.80	30.5		
11077	Colon gl.	"	24.2	25.0	1.01	.2217	7.97	24.5	1.13	.4641
	Adenoca.	"	24.5	16.0			8.98	25.8		
11384	Colon gl.	"	33.2	22.9	1.38	.0013	7.85	24.6	1.54	.0011
	Adenoca.	"	45.8	17.4			12.10	25.6		
45-48	Rectum gl.	"	18.1	24.8	2.18	.0000	6.77	25.8	1.77	.0000
	Adenoca.	"	39.6	22.0			12.00	25.6		
45-142	Rectum gl.	"	14.0	34.3	2.28	.0000	5.30	25.2	1.35	.0294
	Adenoca.	"	32.0	19.4			7.14	30.1		
11223	Bronchial ep.	"	24.2	27.1	1.05	.3669	7.45	36.0	1.53	.0040
	Adenoca.	"	25.4	27.0			11.42	26.6		
11375	Bronchial ep.	"	45.6	11.5	.98	-.4090	5.90	42.9	1.86	.0000
	Adenoca.	"	44.6	23.3			10.98	30.1		

See text for explanation of headings for table.

their preparation and staining, in intensity of the light source, in the amplifier, and for the absorption of the light by unstained tissue and endogenous pigments. For instance, the measurements on the primary carcinoma of the liver were corrected for the light absorption of unstained hepatic cells as well as for that of bile pigments (Fig. 1). The details of these corrections, the operation of the photometric apparatus, and the calculation of the absorption measurements per unit volume of tissue and per cell have been described (29). It should be emphasized that this photometric histochemical method permits one to visualize the exact cells to be measured. Artifacts, due to (a) foreign material; (b) other types

The field of tissue to be measured was determined by a diaphragm in the eyepiece of the microscope. For relatively homogeneous tissues the ocular diaphragm delimited a stage field of 36×55 microns; for less homogeneous tissue a second diaphragm exposed a smaller area, measuring 16×22 microns. "Since in the areas measured the light passed through a uniform thickness of tissue, the results of the absorption per area may be correctly considered as the absorption of a definite volume of tissue" (29). These differences in the unit of tissue volume measured are indicated as "large" and "small" in Table II. Comparisons between tissues measured with oculars of different sizes should not be attempted.

The number of cells in each field measured was recorded and used in the calculation of the mean absorption per cell. On each type of tissue measurements were made on 50 areas in each of 2 adjacent serial sections of 7 microns thickness. The mean values were computed and analyzed statistically for their variation and significance.

The volumetric ratios of the nucleus, cytoplasm, nucleolus, chromatin and nuclear sap were determined by an adaptation of the method described by Chalkley (7). The basophilic material of the nuclear membrane was counted as chromatin. At least 3,000 hits were recorded on each specimen, and the results obtained could be readily duplicated. The ratios were recorded simultaneously on counting equipment designed for differential blood counts. In specimens in which the chromatin was dense or the nucleolus indistinct, only the nuclear-cytoplasmic ratio was calculated. This method of determining proportions of cellular constituents is more accurate than others (29). Instead of expressing the figures in terms of ratios, which may be confusing, the results were recalculated and expressed as percentages of the total cellular or nuclear volume. Relative values for mean cellular volume were obtained by dividing the mean number of cells per field into the arbitrary figure 100 for the tissues measured with the large diaphragm and into 18 for the small diaphragm, these being proportional figures for the unit volumes measured.

In contrast to the satisfactory method of staining for desoxyribose nucleic acid, there is no good histochemical method for demonstrating ribose nucleic acid. A number of investigators have described the estimation of ribose nucleic acid by observation of the decrease in material stainable with pyronin-methyl green or with toluidine blue (1, 2, 12, 13, 17), but even with precautions this method is apt to give misleading results at times (30) for some buffers can extract a large part of the cytoplasmic ribonucleotides. Although cognizant of these difficulties, an attempt was made to get an approximation of the ribose nucleic acid content of some of the tissues.

Six serial sections, adjacent to those stained with the Feulgen method and with hematoxylin and eosin, were used for measurements of ribose nucleic acid. Two adjacent sections were incubated at $50 \pm 1^\circ \text{C}$. in a solution of ribonuclease enzyme¹ in McIlvain's buffer at pH 7.0, two adjacent control sections were incubated under the same conditions in buffer that did not contain ribonuclease enzyme, and two more adjacent sections were left in distilled water at room temperature for the same period. All 6 were then

stained simultaneously with pyronine in the same dish. Tissues that had been fixed in Zenker's fluid were incubated for 3 hours in 1 mgm. ribonuclease per cc. of buffer. Specimens fixed in sublimate alcohol for 2 hours or in formaldehyde for $1\frac{1}{2}$ hours were both incubated in a solution of 0.05 mgm. of ribonuclease per cc. of buffer. The sections were stained for 20 minutes at room temperature in Unna-Pappenheim solution (15, p. 176) from which the methyl green dye had been deleted. They were then rinsed rapidly in water, dehydrated in tertiary butyl alcohol, cleared in xylol, and mounted in clarite. Tertiary butyl alcohol was employed instead of ethyl alcohol because it extracts the dye more slowly and gives a slightly better differentiation (21, 22).

All sections except the control sections left in distilled water were measured with the apparatus under the same conditions as employed for thymonucleic acid; these controls were used only as a visual check on the staining of the other sections. The ribose nucleic acid content of each tissue was calculated by subtracting the mean absorption of light by the tissue treated with ribonuclease from the mean absorption of the tissue incubated only in the buffer. The mean of the 50 volumes on the 2 sections of normal tissue was compared with the corresponding mean for the malignant tumor. In some tissues the pyronine was concentrated so that cell nuclei were not readily counted. Therefore the more accurate mean values for cells per volume obtained with the Feulgen reaction were used in the calculations of all figures for amounts of ribose nucleic acid per cell.

RESULTS

Thymonucleic acid.—The results of the photometric measurements of the absorption of light by the stained thymonucleic acid in the 20 specimens are shown in Table II. In each specimen, the diagnosis of which is given in Table I, measurements were made upon the tumor as well as upon the normal tissue from which such tumor presumably originated. The carcinoma of the liver cell type was compared with the morphologically normal adjacent hepatic parenchymal cells (Figs. 1 and 2). The leiomyosarcoma, which arose in the region of the adrenal and invaded the portal vein, was compared with the smooth muscle of the wall of the surrounding vein. Epidermoid carcinomas were compared with squamous epithelium, basal cell carcinomas with the two basal layers of stratified squamous epidermis, transitional cell carcinomas with transitional epithelium, renal cell carcinomas with epithelium of the convoluted tubules, bronchogenic adenocarcinomas with bronchial epithelium, and other adenocarcinomas with comparable glandular epithelium (Figs. 3 and 4). In specimen

¹ The ribonuclease was furnished by Dr. M. Kunitz, of the Rockefeller Institute, Princeton, N. J.

7362 of the larynx measurements were made of the epidermoid carcinoma, normal stratified squamous epithelium, and normal columnar epithelium. Specimen 45-328 was a turban tumor of the scalp; there is some disagreement as to the cell of origin of this tumor, so the epithelium of the basal layers of the epidermis and the epithelium of the sweat glands were both measured.

For each type of tissue the percentages of absorption per volume of tissue and per cell, coefficients of variation and values for the probability, P , are averaged for each two adjacent serial sections. The figure P is indicative of the statistical probability of such a difference occurring by chance (18, p. 80). For specimen 11336 a P of 0.0000 indicates that there is less than 1 chance in 10,000 of getting the differences shown by the mean absorption measurements of 11.2 and 22.2 per cent. The differences between mean values for which P is greater than 0.01 are not considered statistically significant. For specimens 7362 and 45-328 the values for P refer to the difference between each normal tissue and the tumor. Values for P preceded by a minus sign indicate the probability in instances in which the normal tissue had greater absorption than the tumor.

The ratios shown in Table II are a convenient form of expressing differences in the absorption per unit volume and per cell for normal and malignant tumors. In 18 of the 20 specimens the absorption per unit volume and per cell was greater in the tumors than in the normal tissues. This increase was statistically significant in 11 instances for the volume of tissue and for the amounts per cell in 9 tissues. In 9 specimens the tumor contained at least 50 per cent more thymonucleic acid per unit volume than the normal tissue, and 7 of these had more than twice as much. In no instance did a tumor contain significantly less thymonucleic acid than the normal tissue.

Ribose nucleic acid.—The data obtained from the measurements of ribose nucleic acid are less quantitative and are summarized briefly in Table III. Measurements of ribose nucleic acid were not attempted on 4 specimens. In 7 instances there was no appreciable difference between the absorption of the malignant and normal tissues. In several tumors the ribose nucleic acid content seemed slightly lower than in the normal tissue. It was not felt that statistical analysis of the data was indicated. In the leiomyosarcoma, renal cell carcinoma, adenocarcinoma of the prostate, adenocarcinoma of the bronchus, and possibly one adenocarcinoma of the colon there appeared to be substantially increased amounts of ribose nucleic acid per unit volume and per cell. In one transitional cell carcinoma there was a moderate increase in the

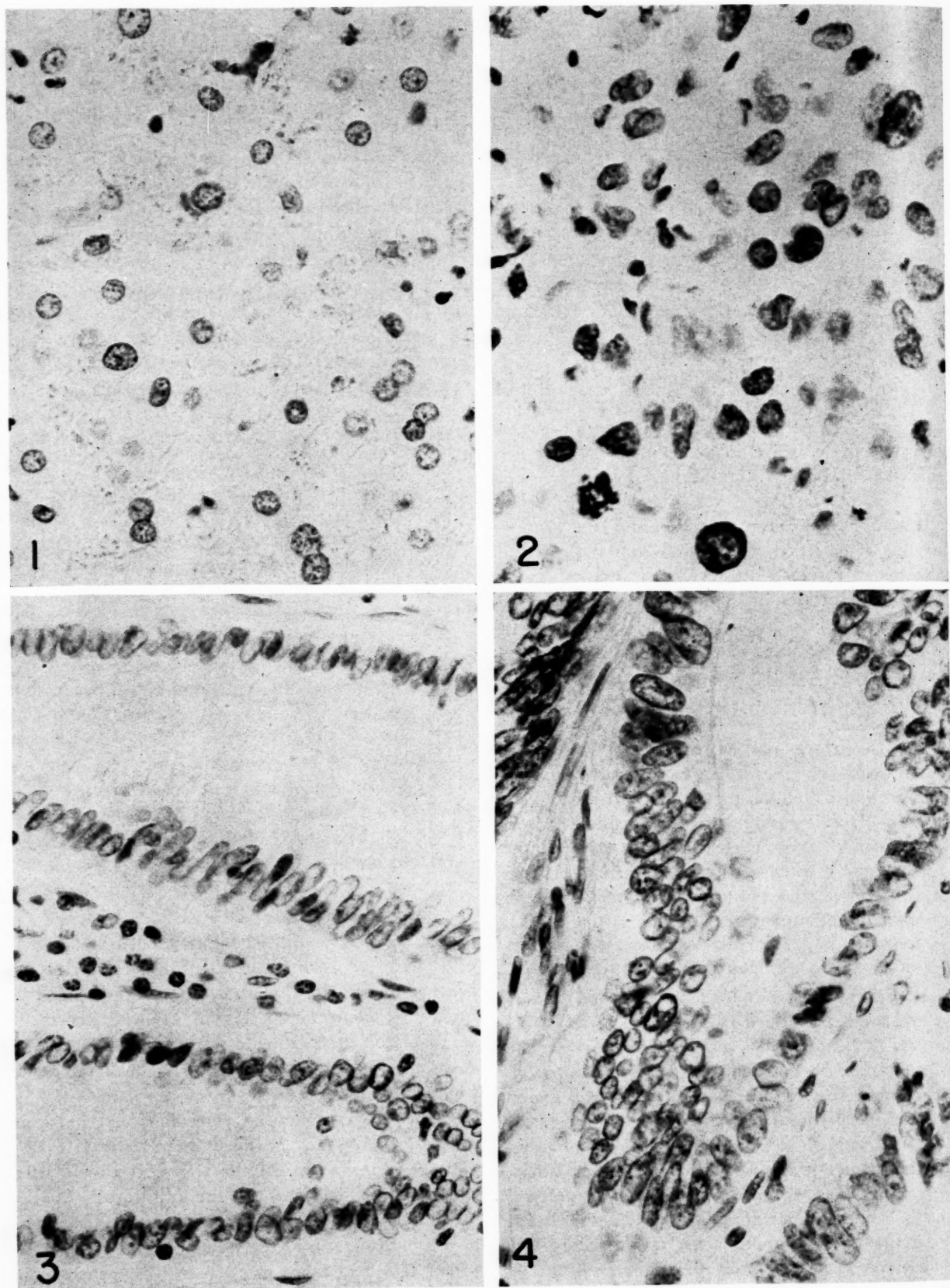
amount per unit volume, and in the other in the amount per cell.

Cellular measurements.—The results of the cellular measurements on each specimen are given in Table IV. The proportion of the total cellular volume occupied by the cytoplasm, nucleus, nucleolus, chromatin, and nuclear sap is expressed in percentile figures. The percentage of the nucleus occupied by nucleolus, chromatin, and nuclear sap is also given. To facilitate comparisons between relative values for comparable tissues, the ratios of the tumor to normal is calculated for the measurements of each constituent. In these ratios the turban tumor, 45-328, is compared with the basal cells of the surface epithelium, and the carcinoma of the larynx, 7362, with the adjacent stratified squamous epithelium.

TABLE III: RATIOS OF RELATIVE REDUCTION IN STAINING WITH PYRONINE OF NORMAL (N) AND TUMOR (T) TISSUES AFTER TREATMENT WITH RIBONUCLEASE

Specimen no.	Light absorption measurement			
	Per volume		Per cell	
	Reduction in % Absorption $T-N$	Ratio T/N	Reduction in % Absorption $T-N$	Ratio T/N
11336	None		None	
11120	8.0	5.0	1.0	3.0
7362	None		None	
5631	6.0	1.5	.1	1.1
10827	-1.4	.9	1.4	1.4
43-939	None		None	
45-328	"		"	
11508	5.8	2.0	2.0	1.9
11364	5.8	1.4	2.8	2.0
11338	-3.0	.7	-1.4	.6
11077	3.6	1.2	2.2	1.4
11384	-3.8	.7	-1.0	.7
45-48	None		None	
45-142	"		"	
11223	"		"	
11375	4.9	1.5	2.2	2.8

The relative amounts, in a given volume, of each of the cellular constituents mentioned above were calculated for each tissue by multiplying the percentage of the constituent by the mean cellular volume. To conserve space, only the results of the ratios of the measurements for each tumor and normal tissue are given in Table V. The difference between these ratios based on relative volume and those in Table IV computed on percentage of cellular and nuclear volume may be illustrated for tissue 6989 in which the mean size of the cells of the epidermoid carcinoma is half that of the cells of the normal squamous epithelium. The tumor tissue contained as much chromatin as the normal (ratio 1.0), but the tumor cells had twice as great a mean concentration of chromatin as the normal cells (ratio 2.05) and the tumor nuclei had mean concentration one-third greater (ratio 1.38).



FIGS. 1-4

TABLE IV: RELATIVE VOLUMES OF CELLULAR CONSTITUENTS, EXPRESSED IN PERCENTAGE OF CELL NUCLEAR VOLUME, AND VOLUMETRIC RATIOS OF TUMOR (T) TO NORMAL (N) TISSUE

Specimen no.	Tissue	Mean cell size	Percentage of cellular volume					Percentage of nuclear volume		
			Cytoplasm	Nucleus	Nucleolus	Chromatin	Nuclear sap	Nucleolus	Chromatin	Nuclear sap
11336	Liver	16.6	87.7	12.3	1.7	7.5	3.1	14.4	59.6	25.9
	Carcinoma	14.6	62.3	37.7						
	T/N	.88	.71	3.06						
11120	Muscle	22.9	89.1	10.9						
	Myosarcoma	12.5	79.2	20.8						
	T/N	.54	.89	1.91						
6989	Epidermis	12.0	75.8	24.2	1.5	15.0	7.7	6.8	61.6	31.6
	Carcinoma	6.3	63.9	36.1	.3	30.7	5.1	.8	85.1	14.1
	T/N	.52	.84	1.49	.20	2.05	.66	.12	1.38	.45
7362	Col. ep.	4.7	57.0	43.0	2.0	32.9	8.1	4.7	76.5	18.8
	Sq. ep.	9.7	73.8	26.2	1.9	17.1	7.1	7.5	65.4	27.1
	Carcinoma	4.7	65.2	34.8	.3	29.1	5.4	1.0	83.5	15.5
	T/Sq. ep.	.47	.88	1.33	.16	1.7	.76	.13	1.28	.57
7306	Epidermis	7.9	71.2	28.8	1.2	24.8	2.8	4.2	85.9	9.9
	Carcinoma	7.9	70.9	29.1	1.8	23.2	4.2	6.3	79.8	13.9
	T/N	1.00	1.00	1.01	1.5	.94	1.5	1.5	.93	1.40
6894	Epidermis	10.0	73.0	27.0	1.8	4.4	20.8	7.0	16.3	76.7
	Carcinoma	7.7	72.4	27.6	1.9	7.6	18.1	7.1	27.5	65.4
	T/N	.76	.99	1.02	1.06	1.73	.87	1.0	1.68	.85
1582	Epidermis	8.1	75.2	24.8	2.1	13.9	8.8	8.5	55.9	35.6
	Carcinoma	6.6	73.6	26.4	2.0	17.6	6.8	7.7	66.4	25.9
	T/N	.81	.98	1.06	.95	1.27	.77	.92	1.18	.73
5631	Trans. ep.	7.3	64.0	36.0	1.3	31.5	3.2	3.6	87.4	9.0
	Carcinoma	5.5	43.3	56.7	.6	54.7	1.4	1.2	96.3	2.5
	T/N	.75	.68	1.58	.46	1.74	.44	.33	1.10	.28
10827	Trans. ep.	4.9	73.2	26.8	1.3	20.2	5.3	4.3	75.4	20.3
	Carcinoma	7.2	65.3	34.7	.4	30.0	4.3	1.1	86.4	12.5
	T/N	1.47	.89	1.29	.31	1.48	.81	.26	1.14	.62
43-939	Basal ep.	4.8	64.2	35.8	.4	33.8	1.6	1.2	94.2	4.6
	Carcinoma	3.5	59.5	40.5	.5	33.7	6.3	1.2	83.3	15.5
	T/N	.73	.93	1.13	1.20	1.00	3.94	1.0	.88	3.37
45-328	Basal ep.	4.2	62.6	37.4						
	Sweat gl.	3.7	74.8	25.2						
	Carcinoma	3.5	58.7	41.3						
	T/Basal ep.	.83	.94	1.10						
11508	Tubules	6.3	63.7	36.3						
	Carcinoma	6.5	58.7	41.3						
	T/N	1.03	.92	1.14						
11364	Prostate gl.	3.4	63.6	36.4						
	Adenoca.	4.8	58.0	42.0	2.7	32.5	6.8	6.4	77.4	16.3
	T/N	1.41	.91	1.15						
11338	Gastric gl.	6.6	55.2	44.8						
	Adenoca.	5.6	59.0	41.0						
	T/N	.85	1.07	.91						
11077	Colon gl.	5.8	65.3	34.7						
	Adenoca.	6.2	55.3	44.7	5.8	30.4	8.5	12.9	68.0	19.1
	T/N	1.07	.86	1.29						
11384	Colon gl.	4.0	64.8	35.2	.9	30.0	4.3	2.6	85.2	12.2
	Adenoca.	4.6	53.9	46.1						
	T/N	1.15	.83	1.31						
45-48	Rectum gl.	6.4	58.8	41.2	3.3	31.4	6.5	8.1	76.2	15.7
	Adenoca.	5.3	49.1	50.8	3.6	35.5	11.7	7.1	69.9	23.0
	T/N	.83	.84	1.23	1.09	1.13	1.80	.88	.92	1.46
45-142	Rectum gl.	6.7	72.6	27.4	.4	23.4	3.6	1.4	85.1	13.5
	Adenoca.	3.8	65.4	34.6	.7	27.1	6.8	2.0	78.4	19.6
	T/N	.57	.90	1.26	1.75	1.16	1.84	1.43	.92	1.45
11223	Bronch. ep.	5.2	65.5	34.5	.9	29.3	4.3	2.6	84.9	12.5
	Adenoca.	7.8	54.3	45.7						
	T/N	1.50	.83	1.32						
11375	Bronch. ep.	2.2	43.2	56.8	.8	49.6	6.4	1.5	87.4	1.11
	Adenoca.	4.4	50.0	50.0						
	T/N	2.00	1.16	.88						
Mean Ratios of T/N		.96	.90	1.32	.73	1.42	1.34	.76	1.14	1.12

To assist in evaluating comparisons of the absorption and cellular measurements in tumor and normal tissues the results are summarized symbolically in Table VI. Absorption measurements for thymonu-

cleic acid that were statistically significant are designated ++, while those not significantly increased or decreased are indicated as + or -. Measurements for ribose nucleic acid that were at least 50 per cent

LEGENDS FOR FIGURES 1 TO 4

(All Mag. $\times 592$)

FIG. 1.—Normal liver (11336). Absorption measurements corrected for bile pigment, as seen in upper central part of photomicrograph. Feulgen reaction.

FIG. 2.—Carcinoma of liver cell type (11336). Nuclei are larger and more deeply staining than normal tissue (Fig. 1). There is more thymonucleic acid per unit volume of tissue and per cell in the carcinoma. Mitotic figure in lower left

part of photomicrograph. Feulgen reaction.

FIG. 3.—Normal rectal mucosa of specimen 45-48. Epithelial cells stained by Feulgen reaction readily differentiated from cells of supporting stroma. Only epithelial cells were measured.

FIG. 4.—Cells of adenocarcinoma of rectum (45-48) have more thymonucleic acid and nuclear sap and less cytoplasm than normal mucosa (Fig. 3). Feulgen reaction.

greater in the tumors are shown as ++, with smaller differences termed + or -. Similarly, tumors with mean cellular measurements more than 25 per cent larger or smaller than normal are given as ++ or --;

TABLE V: RATIOS OF RELATIVE VOLUMES OF CELLULAR CONSTITUENTS OF MALIGNANT AND NORMAL TISSUES

Specimen no.	Cytoplasm	Nucleus	Nucleolus	Chromatin	Nuclear Sap
11336	.62	2.80			
11120	.48	1.04			
6989	.44	.79	.1	1.0	.3
7362	.43	.64	.05	.8	.3
7306	1.00	1.00	1.0	.9	1.5
6894	.77	.75	.5	1.5	.7
1582	.80	.85	.5	1.1	.6
5631	.51	1.19	.3	1.4	.5
10827	1.31	1.92	.3	2.2	1.5
43-939	.68	.82	1.0	.7	2.0
45-328	.77	.94			
11508	.95	1.17			
11364	1.27	1.67			
11338	.92	.77			
11077	.89	1.40			
11384	.96	1.50			
45-48	.68	1.04	1.0	1.0	1.5
45-142	.51	.72	1.0	.6	1.5
11223	1.23	2.00			
11375	2.20	1.83			
Mean Ratio	.82	1.24	.6	1.1	1.0

--; lesser differences as + or -; and ratios of 1.0 as ±.

Twelve of the 20 tumors had cells of smaller mean size than the corresponding normal tissue. This was true in only 3 of 9 adenocarcinomas, in contrast to 8 of the other 10 carcinomas. In three-fourths of the

specimens the tumor had a smaller proportion of cytoplasm and nucleolar material than the normal tissue. In terms of percentage of total cellular volume, the tumor cells had larger nuclei in 17 of 20 instances and more chromatin in 8 of 10. Alterations in other cellular constituents were more inconstant.

From inspection of the data in the tables it is not apparent why the amounts of thymonucleic acid per

TABLE VII: CORRELATION COEFFICIENTS OF ABSORPTION MEASUREMENTS FOR THYMONUCLEIC ACID (TNA) AND CELLULAR MEASUREMENTS

	TNA volume	TNA cell
TNA per cell	.62	
Cell size	-.69	-.50
Per unit volume of tissue		
Cytoplasm	-.70	-.50
Nucleus	-.41	-.26
Nucleolus	-.48	-.11
Chromatin	-.06	-.01
Percentage per cell		
Cytoplasm	-.67	-.51
Nucleus	.67	.51
Nucleolus	-.15	.18
Chromatin	.68	.36

unit volume of tissue and per cell should be increased in a large proportion of tumors. In an attempt to determine whether increased amounts of thymonucleic acid were associated with (a) the relative amounts of cytoplasm, nucleus, nucleolus, or chromatin per unit volume of tissue or (b) with their percentage per cell, correlation coefficients were calculated (18, p. 47), as shown in Table VII. The

TABLE VI: SUMMATION OF THYMONUCLEIC (TNA), RIBONUCLEIC (RNA), AND CELLULAR MEASUREMENTS EVALUATING SIGNIFICANT CHANGES IN TUMORS IN RELATION TO NORMAL TISSUES

Tissue no.						Relative volume					Percentage of cell				
	TNA vol.	TNA cell	RNA vol.	RNA cell	Cell size	Cytoplasm	Nucleus	Nucleolus	Chromatin	Nuclear sap	Cytoplasm	Nucleus	Nucleolus	Chromatin	Nuclear sap
11336	++	++			-	--	++				--	++			
11120	++	+	++	++	--	--	+				--	++			
6989	++	+			--	--		--	+	--	--	++	--	++	--
7362	++	++			--	--		--			--	++	--	++	--
7306	+	+			±	±	±	±	-	++	±	±	++	-	++
6894	++	+			-	-	--	--	++	--	-	+	+	++	-
1582	++	++			-	-		--	+	--	-	+	-	++	-
5631	+	-	++	+	--	-	+	--	++	-	--	++	--	++	--
10827	+	++	-	+	++	++	++	-	++	++	-	++	--	++	-
43-939	+	-			--	--		±	--	++	-	+	+	±	++
45-328	++	+			-	-					-	+			
11508	+	+	++	++	+	-	+				-	+			
11364	-	+	+	++	++	++	++				-	+			
11338	++	++	-	-	+	-					+				
11077	+	+	+	+	+	-	++				-	++			
11384	++	++	-	-	+	-	++				-	++			
45-48	++	++			-	--	+	±	±	++	-	+	+	+	++
45-142	++	+			--	--	--	±	--	++	-	++	++	+	++
11223	+	++			++	+	++				-	++			
11375	-	++	++	++	++	++	++				+	-			
Totals															
Increased	11	9	4	4	4	3	7		3	5		10	2	6	4
Sl. incr.	7	9	2	3	3	1	4		2		2	8	3	2	
Equal					1	1	1	4	1		1			1	
Sl. decr.	2	2	3	2	6	8	5	1	2	1	15	2	1	1	4
Decreased					6	7	3	5	2	4	2		4		2

cellular measurements and corresponding absorption measurements were employed without segregation as to type of tissue. A perfect coefficient of -1 for cell size and thymonucleic acid per unit volume should indicate a definite inverse relation between the measurements of the two variables. Hence a coefficient of -0.69 does indicate considerable inverse correlation. For comparison, the measurements of cell size were correlated with those of relative volume of cytoplasm and nucleus and mean percentage of nucleus per cell, and coefficient values were obtained of 0.98 , 0.55 and -0.66 . The results show that there is a tendency for the amounts of thymonucleic acid per volume to be larger in tissues having small cells and a small mass of cytoplasm as well as cells with large percentages of nuclear and chromatin material.

DISCUSSION

The importance of nucleic acids in normal and neoplastic tissues and the evidence of their disturbance in tumors has been reviewed recently (6, 14, 26). It seems probable that the nucleic acids and nucleoproteins may be important in the formation and action of some enzymes (8).

Recently Schneider (19), using macrochemical methods, compared the nucleic acid content of a mouse lung tumor and rat hepatoma with homologous normal tissue and found the thymonucleic acid content of both tumors much greater than that of the normal tissues. The ribose nucleic acid was increased in the lung tissue and about the same in the hepatoma and normal liver.

Carruthers and Suntzeff (4) studied the extractable desoxyribose nucleic acid in the epidermis of mice before and after painting with methylcholanthrene, and of a transplanted epidermoid carcinoma. Their results are in general agreement with those of Stowell (22), and support the accuracy of the histochemical method. Their selection of the transplanted carcinoma for comparison with normal and hyperplastic epidermis was unfortunate. The original tumor for this transplant was obtained from mice used in experiments by Stowell and Cramer (28). The transplants vary somewhat in their cellular morphology and at times produce considerable keratin. In the earlier work of Stowell (22) in the same laboratory it was shown that one of these transplants had much less thymonucleic acid per unit volume of tissue than the 6 other carcinomas induced by methylcholanthrene. The observations by Carruthers and Suntzeff that these transplanted carcinomas contained less desoxyribose nucleic acid than the mean values for other epidermis confirms the earlier observations by Stowell (22), but should not be considered of significance for tumors in general. Both of these macrochemical investigations (4, 19) support the

histochemical finding of increased amounts of nucleic acids in tumors.

The validity of these histochemical measurements is also based upon the relative specificity of the Feulgen reaction, and the belief that the absorption of complementary monochromatic light by the stained material is proportional to the amount of thymonucleic acid present. The recent assertion by Carr (3) that the Feulgen reaction is not related to nucleic acid lacks adequate experimental proof. The preponderance of work shows that under carefully controlled conditions the Feulgen technic is relatively specific for thymonucleic acid (24, 27). Experiments in this laboratory also indicate that there is a definite relationship between the absorption readings and the amount of nuclear material stained by the Feulgen reaction.

The results with the estimation of ribose nucleic acid by means of the ribonuclease enzyme are less satisfactory (30). The cytoplasmic nucleotides are more unstable and after some types of fixation may be partially extracted by certain buffer solutions. The pyronine stain is not specific, and the ribonuclease enzyme itself shows poor specificity under some circumstances. These difficulties were apparent in preliminary experiments; however, since it was the technic best suited for photometric analysis in the visible light range the method was applied under the most suitable conditions. Because of their less qualitative nature, the results were not subjected to detailed statistical analysis.

The choice of the fixative is of more importance for the ribonuclease technic than for the Feulgen method. Many fixatives destroy the mitochondria in which the cytoplasmic nucleic acids are concentrated (Claude, 9, 10). This would not invalidate the technic unless the nucleates were lost from the cells, because their staining reaction could still be measured after diffusion throughout the cytoplasm.

Statistical analysis of data in these photometric histochemical methods is indicated because the size of the samples is small compared with that employed in macrochemical methods. The results of the chemist who measures millions of cells do not require statistical treatment even though the constituent cells vary greatly, whereas measurements averaging the results in groups of hundreds of these same cells should be analyzed.

In attempting to evaluate the significance of alterations in the nucleic acids of tumors one seeks information as to (a) whether they are increased in all tumors as a specific property of neoplasia; (b) how they are altered in other tissues; (c) whether the changes in nucleic acid content are correlated with any morphologic characteristic of the tissue or with differences in their distribution in the cell; and

(d) whether qualitative changes or functional differences as well as quantitative changes are present. Most of the meager available information included in recent review articles will not be repeated here in detail. Some of the present confused interpretation of results arises from unwarranted attempts to compare research based on diverse methods and materials. The seeming emphasis upon histochemical and cytochemical methods is partially due to the facts that (a) these data are more comparable and pertinent to the present study; (b) the macrochemists have employed methods and materials that often do not lend themselves to correlation with each other, and especially with morphologic cellular data; and (c) certain information can be best obtained by cytochemical methods. Obviously it would be desirable to have correlated studies performed simultaneously on similar tissue, using macrochemical and histochemical methods for both types of nucleic acids.

One of the purposes of this research was to ascertain how frequently different types of tumors have increased amounts of nucleic acids. The results indicate that these are increased in many neoplasms, though perhaps not in all, and certainly not in every cell of every malignant neoplasm. In the 34 specimens analyzed by this photometric histochemical method, including the 20 in this paper, 11 in a previous study of epidermoid carcinomas (28), and 3 in an unpublished investigation, no tumor contained a significantly decreased mean amount of thymonucleic acid per unit volume of tissue or per cell. It is possible that if more cells had been measured the thymonucleic acid might have been significantly increased in even a higher percentage of tumors. However, the evidence now available does not justify a statement that a significant increase in thymonucleic acid is characteristic of all neoplastic cells. Similar observations should be made on additional different malignant and benign tumors and on normal tissues growing at different rates and under different conditions. The role of the protein component of the nucleoproteins of normal and neoplastic cells also should be investigated. The observations of Caspersson and his co-workers (5, 6) indicate that some tumor cells contain increased amounts of ribonucleotides.

Observations on transplantable mammary tumors in rats and mice (23) and unpublished observations on a few human neoplasms with this histochemical photometric method indicate that some tumors have decreased amounts of thymonucleic acid following roentgen radiation. It is not yet established whether this is a constant finding. Mitchell (16) found increased amounts of ribonucleoproteins in the cytoplasm of irradiated cells. Many cells in which there is a high rate of protein formation contain high concentrations of cytoplasmic nucleotides (5, 6). It is

evident that the amounts of both types of nucleic acid vary greatly in different normal tissues and under different physiologic and pathologic conditions.

Another objective of this research was to determine whether increased nucleic acid is a specific property of malignant tissues, and whether the increase can be correlated with any morphologic characteristic. The results indicated a tendency for tissues with large amounts of thymonucleic acid per volume to be composed of small cells with scant cytoplasm. Tissues composed of cells with a high percentage of chromatin had large amounts of thymonucleic acid per volume, but the correlation was not high, perhaps because of the greater and more significant variations in the cytoplasmic content. There is inconclusive evidence in the present study and in earlier work (28) that anaplastic tumors contain more thymonucleic acid. Additional observations are desired, but the present results indicate that many tumors have increased amounts of thymonucleic acids for unknown reasons, or perhaps as a specific property. Caspersson and Santesson (6) have shown that rapidly growing tumor cells have large amounts of cytoplasmic ribonucleoproteins, and suggest that cancer is related to a disturbance in the heterochromatin.

In earlier studies on epidermis (29) it was found that normal stratified squamous epithelium, composed of basal and spinous cells, showed more variation in nucleic acid content than epidermoid carcinomas. In 17 of the present 20 specimens the measurements on the normal tissues had a higher coefficient of variation than those on the corresponding tumor, which would suggest that mean values for tumors are no more misleading than those for normal cells. The mean values are of considerable interest, even though they do not give a comprehensive picture of the variation in the data. Because of the technical difficulties, measurements of mean volume of nucleoli or chromatin are less accurate than those of nuclei.

Qualitative and functional studies of the nucleic acids in tumors is an important yet virtually unexplored field. Additional work will be necessary to elucidate the role that nucleic acids and nucleoproteins may play in the cause of cancer. Histochemical and cytochemical observations permitting the correlation of cellular chemistry and morphology in small identified samples is one of the most important methods of approach to this problem.

SUMMARY AND CONCLUSIONS

Twenty human tumors were analyzed for their content of desoxyribose and ribose nucleic acids. The nucleic acids were measured in sections of tissue with a special photometric instrument consisting of a stable light source, filters, microscope, photocell, and ampli-

fication and recording apparatus. The relative amounts of desoxyribose nucleic acid in adjacent normal and neoplastic tissues were measured by determining the absorption of monochromatic complementary light in Feulgen-stained material. Similarly, but less satisfactorily, the ribose nucleic acid was estimated by measuring the decreased staining with pyronine after treatment with ribonuclease enzyme. This photometric histochemical method has the advantage that the exact cells being measured are visualized and identified as one type. The results, expressed as mean amounts per unit volume of tissue and per cell, can be compared with mean volumetric measurements of the cell, cytoplasm, nucleus, chromatin, nucleolus, and nuclear sap.

In 18 of the 20 tumors the amount of thymonucleic acid per unit volume and per cell was greater than in the adjacent homologous normal tissue. Statistical analysis showed that this increase per unit volume of tissue was significant in 11 instances and per cell in 9 tissues. Two-thirds of the tumors had more ribose nucleic acid per unit volume and per cell than the corresponding normal tissue, and in half of them this increase was more than 50 per cent. A majority of the tumors had cells of smaller mean size, with less cytoplasm. In most instances the tumor cells had larger mean percentages of nuclear and chromatin material. Correlation coefficients show that the amounts of thymonucleic acid are larger in tissues having small cells and small masses of cytoplasm as well as cells with large percentages of nuclear and chromatin substance.

In a total of 34 tissues so far analyzed by this method none have contained a statistically significant decrease in thymonucleic acid per unit volume. These results lend further support to the theory that tumors have disturbances in their nucleoprotein and enzyme systems.

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Multiple Malignant Growths*

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In recent years a number of articles have been published on the subject of multiple primary cancers. The studies of Warren and his associates (6, 7, 8) indicate that the incidence of multiple primary cancers is sufficiently high to presuppose increased individual susceptibility. On the other hand, Peller (4, 5) has suggested that a cured cancer protects against the development of other malignant neoplasms, and has even advocated the experimental induction of a skin cancer to prevent subsequent occurrence of the more fatal cancers of other organs.

Obviously this is a question of considerable importance. If the theory that a cured tumor gives "immunity" were found to be correct, the feasibility of active "immunization" against cancer by this means would have to be considered. If the hypothesis of increased susceptibility of the cancerous individual to more than one cancer proves to be correct, the genetic-constitution hypothesis would be strengthened. In fact, the lines for future investigation may be influenced considerably by the acceptance of one or the other of these theories.

In 1943 Lombard and Warren (3) reported that more individuals were found to have multiple malignant growths than would be expected by chance (7). The validity of this finding has been questioned on the grounds that (a) skin cancer was classified by itself and not combined with lip cancer as was done by Peller; and (b) an erroneous conclusion may have been drawn because of mixed classification. This paper has been prepared to clarify these points and to test further the hypothesis that the occurrence of skin cancer "immunizes" individuals against other cancers.

In this study the data used comprised Massachusetts death records, New York State morbidity records (1), and records of patients who attended the Massa-

chusetts Cancer Clinics through 1927-37. The records of the Massachusetts Cancer Clinics furnished the number of known multiple cancers that occurred following the first clinic visit. These were divided into 34 groups according to sex and selected sites of cancer. Persons who died in the calendar year of the first visit or the year following, as well as those alive on July 1, 1944, were omitted from the computations, since it was felt that this would give a more accurate picture than would be obtained if the entire group were included. Those who died shortly after clinic admission would have had, in some cases, multiple

TABLE I: MULTIPLE MALIGNANT TUMORS DISCOVERED AT DEATH

Location	Number of tumors	Number discovered at death	Rate per 100 discovered at death
Skin—Skin	190	3	1.6
Skin—Other sites	143	82	57.4
Other sites—Skin	28	0	0.0
Other sites—Other sites	67	27	40.3
Total cases	428	112	26.2

TABLE II: PERSONS WITH MULTIPLE MALIGNANT TUMORS
RATE PER 100

Location	Dead	Living and dead
Skin—Skin	3.6	4.7
Skin—Other sites	4.2	2.8
Other sites—Skin	0.5	0.9
Other sites—Other sites	2.0	1.5
Total	10.3	9.9
Persons studied	2,981	5,078

cancers that would have been recorded as metastases rather than as new tumors. It was found that in approximately one-half of the individuals with multiple cancers other than skin the presence of a second primary cancer was not known until death (Tables I and II). Therefore the omission of the living patients furnishes a more accurate figure on the frequency of multiple primary malignant tumors.

The number of multiple cancers in each of the subdivisions was compared with an expected number¹ computed by use of person-years obtained from the clinic data, attack rates from the Massachusetts death records, and the New York morbidity records (Table III).

¹ Method of computing an expected number described later.

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TABLE III: OBSERVED AND EXPECTED METACHRONOUS MALIGNANT GROWTHS FOLLOWING PRIMARY MALIGNANT GROWTHS DIAGNOSED AT THE MASSACHUSETTS CANCER CLINICS 1927-37 AMONG PATIENTS WHO DID NOT DIE IN THE CALENDAR YEAR OF THE FIRST VISIT OR THE FOLLOWING YEAR

DEAD JULY 1, 1944												LIVING AND DEAD JULY 1, 1944	
Primary cancers	Metachronous cancers	N	Persons with meta- chronous cancers	Observed metachronous cancers	Expected metachronous cancers	Difference between observed and expected values	$\frac{\text{Observed} - \text{Expected}}{\sqrt{\text{Observed} + \text{Expected}}}$	Difference between ob- served and expected values if expected is		N	Difference between observed and expected values		
								30% greater than estimated	30% less than estimated				
Male													
Skin		745	159	234	57.0	177.0	10.4	159.8	194.2	1,348	309.3		
	Skin		73	133	10.5	122.5	10.2	119.3	125.7		281.2		
Lip	Lip		6	7	2.2	4.8		4.1	5.5		10.4		
	Other sites		80	94	44.3	49.7	4.2	36.4	63.0		17.7		
		227	33	45	15.5	29.5	3.8	24.7	34.2	481	27.7		
	Skin		6	15	2.9	12.1	2.9	11.2	13.0		19.3		
Other Sites	Lip		4	5	0.4	4.6		4.4	4.7		8.5		
	Other sites		23	25	12.2	12.8	2.1	9.1	16.5		0.1		
		445	21	27	16.4	10.6	1.6	5.7	15.6	610	14.8		
	Skin		8	10	2.5	7.5		6.7	8.3		22.4		
	Lip		1	2	0.6	1.4		1.3	1.6		1.4		
	Other sites		12	15	13.3	1.7		-2.3	5.7		-9.0		
Female													
Skin		435	74	99	32.0	67.0	5.9	57.3	76.7	903	108.0		
	Skin		34	57	4.5	52.5	6.7	51.1	53.9		120.3		
Lip	Lip		0	0	0.1	-0.1		-0.1	-0.1		-0.1		
	Breast		7	7	5.6	1.4		-0.3	3.1		-5.0		
	Uterus		5	5	3.9	1.1		-0.1	2.3		-2.5		
	Other sites		28	30	17.9	12.1	1.8	6.7	17.5		-4.7		
		13	3	3	0.9	2.1		1.8	2.4	29	1.0		
	Skin		1	1	0.1	0.9		0.9	0.9		0.8		
Breast	Lip		0	0	0.0	0.0		0.0	0.0		0.0		
	Breast		0	0	0.2	-0.2		-0.3	-0.1		-0.4		
	Uterus		0	0	0.1	-0.1		-0.1	-0.1		-0.3		
	Other sites		2	2	0.5	1.5		1.3	1.7		0.9		
		593	9	9	12.9	-3.9	0.8	-7.8	0.1	858	-2.9		
	Skin		1	1	1.3	-0.3		-0.7	0.1		6.1		
Uterus	Lip		0	0	0.0	0.0		0.0	0.0		0.0		
	Breast		1	1	1.5	-0.5		-1.0	0.0		1.0		
	Uterus		3	3	2.3	0.7		0.0	1.4		-0.3		
	Other sites		4	4	7.8	-3.8	1.1	-6.1	-1.4		-9.7		
		303	5	5	5.6	-0.6		-2.3	1.1	493	-2.6		
	Skin		0	0	0.5	-0.5		-0.7	-0.3		1.2		
Other Sites	Lip		0	0	0.0	0.0		0.0	0.0		0.0		
	Breast		2	2	1.4	0.6		0.2	1.0		1.6		
	Uterus		0	0	0.1	-0.1		-0.1	-0.1		-0.3		
	Other sites		3	3	3.6	-0.6		-1.7	0.5		-5.1		
		220	5	6	7.7	-1.7		-3.9	0.7	356	-0.8		
	Skin		0	1	0.9	0.1		-0.2	0.4		4.7		
Totals	Lip		0	0	0.0	0.0		0.0	0.0		0.0		
	Breast		1	1	1.5	-0.5		-1.0	0.0		-0.3		
	Uterus		1	1	1.1	-0.1		-0.4	0.2		-0.2		
	Other sites		3	3	4.2	-1.2		-2.3	0.1		-5.0		
		2,981	309	428	150.1					5,078			

Only 4 of the 34 groups studied showed a significant difference between observed and expected values. These were "skin-skin" in both sexes, "skin-other sites" and "lip-skin" among males. If the sites of the metachronous cancers were ignored, the skin cancers of both sexes and the lip cancers of males were followed by more observed metachronous cancers than would be expected. While it is believed that the computed expected values are close to the true figure, the possibility of error exists. A 30 per cent difference in the expected values would lie well beyond any such error. Neither increasing nor decreasing the expected values by 30 per cent altered the fact of significance of the differences, but decreasing the expected by 30 per cent added "lip-other sites" among males and "skin-other sites" among females to the significant groups.

Supplementary comparisons were made using records of both the living and the dead combined. While this group is known to furnish less accurate information than the dead group, it did show considerable similarity. "Skin-skin" cases of both sexes, "skin-lip," "lip-skin," "lip-lip," and "other sites-skin" of males showed significances.

In all instances where significant differences occurred, the observed values exceeded the expected. The "skin-skin" combination showed definite significance and indicated a strong predisposition to multiple malignant growths. There is also an indication that males with lip cancers have some predisposition to multiple cancers. Immunity to the formation of a second primary cancer is not produced by the presence of a skin cancer, and while in many of the sites the figures are too small to draw conclusions there is nothing to suggest that cancer at any site produces "immunity."

DISCUSSION

CHOICE OF DATA

The observed values were the total known cancers that presumably developed after clinic admission among persons who died prior to July 1, 1944, rather than the number of persons with multiple cancers. The total number of multiple cancers was used in order to permit a better computation of the expected values. If the data had been based on the individual patients, it would have been necessary to limit the use of person-years to the period between clinic admission and the appearance of the first metachronous cancer. In many cases it was impossible to determine the correct interval.

Records of persons who had died were chosen, rather than a combination of the living and dead, for two reasons. First, their life span was completed and they could not develop a multiple cancer later. The

second and more important reason was the fact that in over half of the patients with multiple cancers other than skin, the development of the second cancer was not known to the clinic until after death. Patients with multiple skin cancers usually returned to the clinic, but many of those with cancers of other organs did not. It seemed reasonable to conclude that among the living group there might be many with metachronous cancers, and a study of the dead alone would be preferable if it could be proved that this selection did not furnish an erroneous measure of metachronous cancers.

A study based on the dead only might be criticized on the grounds that autopsied cases might affect the figures, and that the presence of a second cancer might have hastened death. Apparently neither of these suppositions was of great importance in this series. A review of the cases showed that among patients with single malignant growths 7.8 per cent were autopsied, while among those with multiple malignant growths 9.2 per cent were autopsied—a difference of 1.4 ± 1.8 .

The other premise, the possible hastening of death by the presence of a second cancer, was found to be negligible by means of the following computations. The average duration of life from the first clinic visit to death for persons with single cancers and those with multiple cancers was compared. These durations were $6.80 \pm .17$ years for patients who died with multiple cancers and $4.90 \pm .05$ for those who died with only one cancer. Inasmuch as many of the cancers were of the skin, and as the probability of a skin cancer hastening death might be expected to be less than that of a cancer at another site, new durations were obtained for both groups omitting the records of persons in which skin cancer was present either alone or as one among multiple cancers. The averages were $6.40 \pm .39$ for multiples, $4.36 \pm .06$ for singles. It was suggested that the two groups might not be comparable in respect to age-sex, duration of disease prior to clinic visit, and distribution in time over the years of observation. The groups did not differ statistically from each other as to time of first clinic attendance or in duration of disease prior to clinic visit. There was a difference between the groups as to the age distribution of females. Age-sex adjustments were made and the resulting durations showed $6.62 \pm .40$ for multiple and $4.42 \pm .06$ for single cancers. The multiple cancer group persists in having the longer duration. This finding was not anticipated and was the reverse of what might have been expected. It indicates that in this study hastening of death by multiple cancers did not occur.

In order to eliminate more completely the effects of early death from severe malignant neoplastic disease, durations were computed for persons who died more than 5 years after their first clinic visit. Two such were calculated: for single cancers, and for multiples in which the second cancer was not of the skin. The results were identical; 9.4 years.

Further computations were made for males with one skin cancer and for those with multiple cancers in the "skin-other sites" group. The duration for skin cancers alone was $5.81 \pm .13$ years, for "skin-other sites" $5.52 \pm .31$ years. The difference is not significant.

The opinion seems justified that any effect a second cancer may have had in hastening death in this series of cases is so slight that it may be ignored.

COMPUTATION OF EXPECTED VALUES

Person-years were computed from the date of the original clinic visit either to date of death or, in the case of the living, to July 1, 1944. Expected values were computed by applying to the person-years age-sex-site specific incidence rates. These were the mortality rates for the centering point of the person-years increased by the cure rates and reduced by a correction factor when necessary.

This correction factor was required because the probability of another independent cancer, assuming independence, would be less since multiple cancer could not occur in a site already destroyed. With the skin this would have little effect, because the area involved by the primary cancer would be small in relation to the total area of the skin; with the lip

TABLE IV: EXAMPLE OF METHODOLOGY IN OBTAINING EXPECTED NUMBER OF METACHRONOUS CANCERS

Age	Male skin person-years	Average, male skin cancer age specific death rates, per 100,000 population (3-year average centering on 1937)	Columns 1 \times 2	Expected male skin cancers ($\frac{\text{Column 3}}{10} \times 100$)
	(1)	(2)	(3)	(4)
30-34	2.00	0.21	0.000004	0.00004
35-39	5.00	0.44	0.000022	0.00022
40-44	37.00	0.68	0.000252	0.00252
45-49	88.00	1.18	0.001038	0.01038
50-54	182.00	1.85	0.003367	0.03367
55-59	313.00	3.56	0.011143	0.11143
60-64	485.50	5.99	0.029081	0.29081
65-69	736.75	11.30	0.083253	0.83253
70-74	987.25	9.80	0.096751	0.96751
75-79	900.00	25.62	0.230580	2.30580
80 and over	630.25	94.67	0.596658	5.96658
Expected number of male skin cancers				10.52149

Lip cancers were not combined with skin cancers, as it is the belief of the authors that such a grouping is not consistent with sound pathology. Skin carcinoma is made up of two chief subtypes, the basal cell group and the epidermoid. Basal cell cancers, which make up about 70 per cent of skin cancers, practically never metastasize and have a mortality rate of about 10 per cent; indeed some observers give as low as 5 per cent. Epidermoid cancers of the skin, roughly 30 per cent of the total, not infrequently metastasize and have about 35 per cent mortality.

Cancer of the lip is a disease of mucous membranes, not of the skin. It is almost always an epidermoid carcinoma and is a much more dangerous disease than cancer of the skin. Its mortality rate, in skilled hands, is 30 per cent. Metastasis to regional lymph nodes occurs in 20 per cent of all cases, and in this group only 50 per cent are well for 5 years.

it might appreciably alter the probability; with the breast at least one-half of the future probability would be lost; and with such organs as the cervix, all of it. No correction factor was used for skin cancers. The expected incidence rate for lip cancer was reduced by one-quarter, breast by one-half, uterus by nine-tenths, and all other sites by one-twentieth.

For skin, lip, and other sites in males, and skin, lip, breast, cervix, and other sites in females, age-sex specific mortality rates were obtained from a 3-year average of deaths centering on the central point of the person-years. For the dead group this was 1937, for the living and dead group 1941.

The mortality rate was changed to an incidence rate by dividing it by 100 per cent minus the percentage of cures and multiplying by 100. This was done on the assumption that the incidence rate would be the death rate plus the cure rate.

The cure rates were based on composite unpublished estimates of several authorities and computations from the New York morbidity data (2). The percentages of cures were estimated to be: skin 90; lip 70; male, other sites 5; uterus 25; breast 25; female, other sites 4.

An example of the methodology of computing expected values is shown in Table IV.

SUMMARY AND CONCLUSIONS

Expected values obtained by multiplying person-years by the age-sex-site specific incidence rate and reduced when necessary by correction factors furnished values that are believed closely to approximate the true values. The fact that similar results can be obtained even when allowing for an error in expected values far greater than would appear to be possible warrants further confidence in the conclusions.

Persons with skin cancers are predisposed to other skin cancers. There is also an indication that males with lip cancers have some predisposition to multiple

skin cancers. There is no evidence that immunity to the formation of a second primary cancer is elicited by the presence of a skin cancer.

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Abstracts

Reports of Research

Induction of Mammary Cancer with Methylcholanthrene. I. Histogenesis of the Induced Neoplasm. KIRSCHBAUM, A., WILLIAMS, W. L., and BITTNER, J. J. [Univ. of Minnesota Med. Sch., Minneapolis, Minn.] *Cancer Research*, 6:354-362. 1946.

When the skins of forced-bred female mice of certain genetic types were painted with methylcholanthrene dissolved in benzene, they developed grossly visible, multiple nodules in the mammary gland. Forced breeding alone did not induce this type of nodule formation. These nodules are considered to be carcinogen-induced, and they developed in either the presence or the absence of the milk agent. Although in whole mount preparations, as viewed under low magnification, the nodules resembled the "hyperplastic nodules" which precede "milk agent tumors," sections of the carcinogen-induced nodules revealed a decidedly different histology. The nodules developed as a result of injury to the epithelium of the ducts and proximal alveoli by the carcinogen, followed by a proliferative epithelial response. These alterations were succeeded by neoplasia. The frank cancers, which developed from carcinogen-induced nodules, were of mixed squamous and alveolar structure. Susceptibility to the carcinogenic induction of mammary cancer is not common to all strains, and this susceptibility cannot be correlated with susceptibility to spontaneous mammary cancer. The histological evidence does not favor the concept that the carcinogen accelerates the sequence of alterations seen in the histogenesis of spontaneous "milk agent tumors" of mice.—Authors' abstract.

Carcinogens and the Regeneration Patterns after Injury. HOWES, E. L. [Coll. of Physicians & Surgeons, Columbia Univ., New York, N. Y.] *Cancer Research*, 6:298-310. 1946.

Carcinogens destroy reticulin and collagen. This destruction could be seen both when the carcinogen was painted on the skin of rats and mice and also when a thread containing the carcinogen was buried in the muscle. Leukocytes and giant cells were also destroyed but epithelial cells and fibroblasts survived the injury and proliferated in response to it, especially after the damaged reticulin and collagen were absorbed. Following this the epithelial cells and fibroblasts grew distortedly in sheets and developed into malignant tumors. The absorption of collagen and reticulin and their failure to regenerate could explain the long latent period between the initial application of the carcinogen and the development of the tumor. No evidence could be found to justify the theory that the

carcinogen stimulated cells directly into the formation of a tumor or that the rate of proliferation exceeded any other form of injury. The leukocytes of the guinea pigs studied, phagocytized the carcinogen, a fact which offers a possible explanation of the resistance of the species. A plea is made for more careful observation of changes in reticulin and collagen in the study of neoplasms.—Author's abstract.

The Carcinogenicity of Wood Soot from the Chimney of a Smoked Sausage Factory. SULMAN, E., and SULMAN, F. [Hebrew Univ., Jerusalem, Palestine] *Cancer Research*, 6:366-367. 1946.

Thirty-six female rats implanted subcutaneously with fragments of soot from the chimney of a sausage factory developed sarcoma in 16.6% of the cases. No tumor developed in 36 male rats implanted intrascrotally with bits of the same soot. Ten female mice treated for 2 years with an ether and alcohol extract of wood soot showed tumor formation in 3 cases (2 sarcomas and 1 carcinoma). Twenty rats fed for 2 years on a diet containing an unlimited amount of smoked sausage failed to develop tumors.

The conflicting finding, carcinogenic effect in parenteral treatment versus absence of carcinogenic effect after oral administration, indicates the need for further study of the carcinogenic activity of smoked food, in view of the practical importance of the problem for human nutrition.—Authors' summary.

Tissue Changes in Experimental Mice Treated with Pentose Nucleotides. BARKER, G. R., GULLAND, J. M., and PARSONS, L. D. [Bernhard Baron Inst. of Path., The London Hosp., and Univ. Coll. Nottingham, London.] *Nature, London*, 157:482-483. 1946.

Adenylic (I), guanylic (II), cytidylic (III), and uridylic (IV) acids were injected into mice (C57, CBA) grafted with homologous methylcholanthrene sarcomas. I and II had an inhibitory action on the growth of these tumors while III had little or no effect. On the other hand IV had a stimulating action. Details are given concerning the effects of these compounds upon the size of the spleen and on the number of giant cells in it. Stock or pure line mice treated with I or III showed a leukocytosis; the absolute numbers of lymphocytes, polymorphs, and immature myeloid cells were increased and the lymphocytes were often more numerous than the polymorphs. A rise in the eosinophils was infrequent and did not exceed 6%. Amyloid infiltration of the spleen and liver followed treatment with I and II but not with III. Mice treated with II

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showed no, or only moderate, leukocytosis while the eosinophils rose from 12 to 16% (normal 1 to 3%). I and II produced reticulosis and plasmacytosis of lymph nodes.

The systemic effects of the growth of primary and grafted sarcomas in mice include leukocytosis, with increase of myeloid cells and crossing of the lymphocyte line by the polymorphs, reticulosis and plasmacytosis of the lymph nodes and amyloid infiltration of spleen and liver. These results suggest that nucleotides may be liberated during the development of primary and grafted sarcomas.—E. L. K.

The Effects of Suramin (Germanin), Azo Dyes, and Vasodilators on Mice with Transplanted Lymphosarcomas. WILLIAMS, W. L. [Yale Univ. Sch. of Med., New Haven, Conn., and Louisiana State Univ. Sch. of Med., New Orleans, La.] *Cancer Research*, **6**:344-353. 1946.

Sixty-one C3H mice received subcutaneous transplants of an estrogen-induced lymphosarcoma and were killed 21 days later. During the terminal 7 to 16 days of this period, 34 mice were injected (subcutaneously) daily with 1 mgm. (0.1 cc. of an aqueous solution) of one of the following: suramin, 20 animals; trypan red, 4; trypan blue, 3; chlorazol fast pink, 7; histamine dihydrochloride, 6. Four animals received 0.1 cc. of depropanex in the same manner.

The azo-dyes had no effect upon the growth or morphology of the transplanted lymphosarcoma but prolonged the bleeding and clotting times. The vasodilators (histamine and depropanex) did not alter the amount or pattern of tumor growth. In these animals and in the untreated controls the weights of the tumors ranged from 7 to 16 gm. (average 10.9 gm.).

In the suramin-treated animals the tumors weighed 1 to 7 gm. (average 3.5) and the inhibition of tumor growth apparently resulted from necrosis of neoplastic lymphocytes. Many of these necrotic cells as well as remnants of them were observed in the tumors. Cytologically normal tumor cells were also present. In the lymph nodes and spleens of suramin-treated animals there was an obvious diminution in the number of lymphocytes. The usual lymphoid areas of these organs consisted almost entirely of reticular cells, macrophages, fibroblasts, large lymphocytes and plasma cells. The lymph nodes showed less actual necrosis of lymphoid elements *in situ* than did the tumors. Bleeding and clotting times were prolonged in the suramin-treated animals and in most cases there was renal damage. Tissue from 2 tumors which grew in suramin-treated animals was successfully transplanted to other C3H mice.

Smaller doses of suramin given to an additional group of ten C3H mice during the immediate interval subsequent to transplantation did not significantly inhibit the eventual growth of the tumor.—Author's abstract.

Quantitative Studies on the Latent Period of Tumors Induced with Subcutaneous Injections of the Agent of Chicken Tumor I. I. Curve Relating Dosage of Agent and Chicken Response. BRYAN, W. R. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **6**:225-237. 1946.

In a series of experiments with partially purified chicken tumor I agent, injected subcutaneously into several breeds

of chickens, it was found that a linear relationship existed between the reciprocal of the latent period of tumor development and the logarithm of the dosage of agent injected. This relationship held, except in an occasional discordant chicken group, throughout a ten million fold dilution of the chicken tumor agent. Under controlled conditions, therefore, it is felt that the latent period is a satisfactory criterion of biological activity of the chicken tumor agent. Employing statistical methods based on this dose-latent period relationship, evidence of heterogeneity among chicken groups was obtained.—R. A. H.

On the Anatomical Character of the Infectious Myxoma of Rabbits. AHLSTRÖM, C. G. [Path. Inst., Lund, Sweden] *Acta path. et microbiol. Scandinav.*, **17**:377-393. 1940. Description.—M. H. P.

Genetic Analysis of the Induction of Tumors by Methylcholanthrene: XII. The Effects of Selection Toward Resistance. STRONG, L. C. [Yale Univ. Sch. of Med., New Haven, Conn.] *Yale J. Biol. & Med.*, **18**:145-155. 1946.

Further evidence is presented to show that the two phenomena, (1) segregation and recombination of genes and (2) mutations or other suddenly acquired biological alterations, are both effective in changing susceptibility to local tumors induced by methylcholanthrene. This is true when the carcinogen has been administered to both parents over a period of several generations of hybrid mice (from the F_4 to F_{12}) and a regime of continued selection toward resistance to such local tumors is consistently employed.

Hybrid mice of 6 separate lines of descent were injected subcutaneously at 60 days of age with 1 mgm. of methylcholanthrene. These lines were continued through 4 to 6 generations by a rigid regime of selection toward resistance to the appearance of the expected local tumors at the site of the injection. No discrimination was practiced in the different lines. Diversified effects of selection were obtained as follows: (1) no effect in changing tumor susceptibility in one subline; (2) an intermediate or rapid decline in the incidence of local tumors in the succeeding generations in 4 sublines, and (3) a gradual shift toward increased local tumor susceptibility counter to the trend of genetic selection. Earlier data of the author have previously disclosed the appearance of a sudden increase of local tumor susceptibility in another subline of methylcholanthrene-injected hybrid mice. The conclusion is drawn that the two phenomena referred to above are involved in shifting susceptibility to local tumors induced by methylcholanthrene.—J. L. M.

Degradation of Cystine Peptides by Tissues. IV. Dehydropeptidase Activity in Normal and Neoplastic Tissues. GREENSTEIN, J. P., and LEUTHARDT, F. M. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **6**:197-201. 1946.

The authors have reported previously that aqueous extracts of liver, kidney and pancreas contain dehydropeptidase activity when chloracetyldehydroalanine is used as a substrate whereas extracts of spleen, brain, muscle and a variety of neoplastic tissues are nearly inactive as far as the hydrolysis of this substrate is concerned. The present investigations reveal, however, that when glycyldehydroalanine is employed as a substrate all tissues tested, both

normal and neoplastic, show dehydropeptidase activity and to about the same degree. This would indicate the existence of two separate dehydropeptidase systems, the one acting upon glycyldehydroalanine being tentatively designated as dehydropeptidase I and the other, active in the hydrolysis of chloracetyldehydroalanine, as dehydropeptidase II. The similarity of distribution of exocystine desulfhydrase and dehydropeptidase II is pointed out.—R. A. H.

Enzymatic Hydrolysis of Benzoylarginineamide in Normal and Neoplastic Tissues. GREENSTEIN, J. P., and LEUTHARDT, F. M. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, 6:203-206. 1946.

The activity of the amidase system capable of hydrolysing benzoylarginineamide at the amide linkage with the evolution of ammonia was investigated in whole extracts of several normal and neoplastic tissues. The order of descending activity of the various normal tissues was found to be spleen, liver, kidney, pancreas, brain and muscle. The rate of hydrolysis appeared to be the same for normal liver and for primary rat and transplanted mouse hepatomas. The activity in fetal rat liver was less, and that of transplanted rat hepatoma was greater, than the activity of normal adult rat liver. Similarities of distribution of this amidase and of catheptic protease and nucleodesaminase are discussed.—R. A. H.

Note on Some Aspects of the Effect of Nucleates in Primary and Transplanted Rat Hepatomas. GREENSTEIN, J. P., and CHALKLEY, H. W. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, 6:207-209. 1946.

The activity of the desaminases for ribosenucleate and desoxyribosenucleate and the effect of added nucleate on the dehydrogenase activity in extracts of primary rat hepatomas were investigated. In both respects, primary hepatomas were found to correspond very closely to normal adult liver but to differ considerably from a transplanted hepatoma.—R. A. H.

Enzymatic Activity in Primary and Transplanted Rat Hepatomas. GREENSTEIN, J. P., and LEUTHARDT, F. M. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, 6:211-217. 1946.

Combining data from the literature with new experimental results, a comparative tabulation is given of a large number of components, enzyme systems, coenzymes and vitamins, of extracts of non-neoplastic rat liver (fetal, normal adult and regenerating adult), primary induced rat hepatomas and a transplanted rat hepatoma. These components are classified into six categories according to different patterns of alterations noted in the different types of hepatic tissue, and some of the implications of these alterations are discussed.—R. A. H.

Protective Effect of Thymus Nucleate on the Heat Coagulation of Proteins. CARTER, C. E., and GREENSTEIN, J. P. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, 6:219-223. 1946.

It has been reported previously that the addition of thymus nucleate to tissue extracts protect the proteins of such extracts from coagulation at 100° C. The present experimentation shows the same protective influence of thymus nucleate for purified egg albumin, 1 mgm. of nucleate being sufficient to prevent the coagulation of

600 mgm. of the protein when kept at 98° C for longer than 120 minutes. This protective action of the nucleate is obviated by the addition of 3 to 4 × 10⁻⁵ mols of NaCl, the concentration of NaCl necessary being independent of the concentration of the nucleate. Fresh aqueous extracts of liver were found to possess a natural protection against heat coagulation. Both the natural protection and that imparted by the addition of nucleate were found to decrease as the extracts were allowed to stand at room temperature or upon incubation. Experiments with the effects of desoxyribonuclease upon the protective ability of thymus nucleate suggest that the loss of protection upon standing or incubation is due to the enzymatic degradation of the nucleate. Although protein coagulation did not occur in the "protected" liver extracts, the enzymatic activity of the extracts, at least in the case of several enzyme systems, was destroyed by the heating.—R. A. H.

The Microscope or the Guinea Pig? GREENE, H. S. N. [Yale Univ. Sch. of Med., New Haven] *Yale J. Biol. & Med.*, 18:239-242. 1946.

The author emphasizes the value of heterologous transplants as an aid to the diagnosis and classification of human tumors. The ability to survive in an alien host is not shared by normal adult tissue, benign tumors, or chronic granulomas, and the mere fact that growth takes place immediately identifies the tissue in question as cancer. The value of heterologous transplantation is further emphasized from a diagnostic point of view by the fact that increased differentiation and organization exhibited by the tissue takes place in the foreign host. Completely disorganized tumors often undergo alterations in stromal-parenchymal relationships and reveal an organized structure indicating the tissue or organ of origin. In other instances, as in the present, an increased differentiation of the transplanted cells betrays their proper classification.

In the reported case, the primary diagnosis of hemangioblastoma was made on a purely morphological basis. In contrast to the disorderly anaplastic appearance of the human biopsy specimen (recurrent tumor), microscopic examination of the transplants in the eye of the guinea pig showed a fairly well organized growth of cells whose structure and arrangement were typically those of a chondrosarcoma. At autopsy several months later widespread metastases were found and the classification of the tumor based on histological examination was chondrosarcoma. The author states that the method of transplanting human cancer into the anterior chamber of the guinea pig's eye is extremely simple, requiring no special technical ability, and can be applied in any hospital laboratory.—J. L. M.

Toxin Therapy of Experimental Cancer. The Influence of Protozoan Infections upon Transplanted Cancer. ROSKIN, G. [Univ. of Moscow, Moscow, U.S.S.R.] *Cancer Research*, 6:363-365. 1946.

Cancer cells may be particularly sensitive to certain protozoan endotoxins and bacterial toxins, while normal cells of a given animal species are immune. Some bacterial toxins and protozoan endotoxins in adequate dosages inhibit the development of certain experimental tumors

and cause complete regression of others. Toxin therapy may become one of the methods for treating malignant tumors.—Author's summary.

The Utilization of Intravenously Injected Salt in Normals and in Patients with Cushing's Syndrome before and after Administration of Desoxycorticosterone Acetate. SOFFER, L. J., LESNICK, G., SORKIN, S. Z., SOBOTKA, H. H., and JACOBS, M. [Mt. Sinai Hosp., New York, N. Y.] *J. Clin. Investigation*, 23:51-54. 1944.

This report deals with the findings in 12 normal individuals and 4 patients with Cushing's syndrome. In normal individuals the intravenous injection of salt following the intramuscular injection of a single dose of desoxycorticosterone acetate resulted in a considerable retention of injected salt, above that seen prior to the injection of the hormone. In contrast to these results, patients with Cushing's syndrome showed a pronounced sodium chloride diuresis. Five case reports are presented.—J. L. M.

Clinical and Pathological Reports

Clinical investigations are sometimes included under Reports of Research

DIAGNOSIS—GENERAL

Diagnostic Aspects of Bronchiogenic Carcinoma. MOERSCH, H. J., *Proc. Staff Meet., Mayo Clin.*, 19:357-361. 1944.

This discussion includes the following topics: roentgenographic examination, bronchoscopy, bronchography and tomography. A filling defect or obstruction of the bronchial tree as demonstrated on bronchography or tomography does not necessarily indicate that the obstruction is due to carcinoma. If possible, tissue should be obtained from the lesion itself to establish the diagnosis. Needle biopsies and thoracoscopic examinations are other diagnostic procedures that may be of help. However needle biopsies possess some element of risk and thoracoscopic examinations frequently yield negative results.—J. L. M.

The Significance of Fluid in the Pleural Space: A Study of 274 Cases. TINNEY, W. S., and OLSEN, A. M. *Proc. Staff Meet., Mayo Clin.*, 20:81-85. 1945.

A review of 444 cases is given in which fluid was present in the pleural space and in which a diagnostic thoracentesis was performed. All cases of pleural effusion which developed as a postoperative complication or as the result of trauma were excluded. In 170 of the 444 cases (38%) diagnosis of the underlying disease was not established, although a tentative diagnosis of tuberculosis was made in 58 cases and metastatic carcinoma of the pleura was suspected in 46 cases. The present study on the significance of fluid in the pleural space was limited to the 274 cases in which the cause of this condition was determined.

The etiologic factors responsible for production of pleural fluid are tabulated. There was a low incidence of inflammatory lesions, such as pneumonia and tuberculosis, in contrast to the high incidence of carcinoma. These figures may be explained by (1) the types of cases encountered at the Clinic, which include a relatively large number of cases of carcinoma and lymphoblastoma and (2) the fact that all patients who had received collapse therapy were excluded. The highest incidence of pleural effusion occurred in cases of intrathoracic tumor and of carcinoma of the breast. In 42 of the 141 cases of carcinoma (30%) malignant cells were found in the aspirated fluid. The technic described by McDonald and Broders was used

in studying pleural effusion for malignant cells. In 193 of the 274 cases (70%) the pleural fluid was serous in type, and in 81 cases (30%) it was hemorrhagic. The total number of cases of carcinoma and lymphoblastoma reveal that a malignant process was the underlying disease in 85% of cases of hemorrhagic effusion. In an additional 10%, the cause was congestive heart failure. This observation is of clinical significance because if congestive failure can be eliminated as a cause of bloody effusion in a specific case there is a 95% chance that a malignant process, either carcinoma or lymphoblastoma, is present. Pulmonary embolus was a complicating condition in 5 of the 8 cases of congestive failure in which the fluid was hemorrhagic.—J. L. M.

FEMALE GENITAL TRACT

Indications for Oophorectomy. HODGE, R. H. [Med. Coll. of Virginia, Richmond, Va.] *Virginia M. Monthly*, 72:286-288. 1945.

Radical surgery (bilateral salpingo-oophorectomy and hysterectomy) is indicated in carcinoma of the ovary of moderate or high malignancy and in adenocarcinoma, sarcoma, and solid teratoma of that organ. Simple oophorectomy is recommended for Brenner tumor, fibroma, and other relatively benign growths.—M. E. H.

Fatal Bronchial Asthma Showing the Asthmatic Reaction in an Ovarian Teratoma. THOMSON, J. G. [Med. Sch., King's Coll., and Roy. Victoria Infirmary, Newcastle-on-Tyne, England] *J. Path. & Bact.*, 57:213-219. 1945.

At the postmortem examination the characteristic picture of asthma was found, comprising blockage of bronchi by mucus, eosinophilous infiltration of bronchial mucosa and submucosa, hyaline thickening of the basement membranes, hypertrophy of the bronchial muscles and mucous glands, the presence of mucus in some of the air vesicles, and, in addition, Charcot-Leyden crystals and Curschmann's spirals. The case was one of status asthmaticus in which adrenaline was ineffective in relieving the condition during life. A unique feature was the presence of a typical asthmatic reaction in some epithelium of respiratory type, which was found in an ovarian teratoma. Histological appearances similar to those in the lungs were found.—L. W. P.

The Vaginal Smear in Diagnosis of Carcinoma of the Uterus. GATES, O., and WARREN, S. [Massachusetts State Tumor Diagnosis Service, Harvard Cancer Commission, and New England Deaconess Hosp., Boston; Pondville Hosp. and Dept. of Pub. Health, Walpole; and Westfield State Sanatorium, Westfield, Mass.] *Am. J. Path.*, 21:567-601. 1945.

The practical aspects of the vaginal smear as a procedure for the routine pathologic laboratory were studied in 233 cases. The authors conclude that, while the method has yet to be clearly established as a means of final diagnosis, it is promising in a high degree and may well be of value as a screening test for detecting the existence of cervical or endometrial cancer in large groups of women.—J. G. K.

Carcinoma of the Endometrium. MEIGS, J. V. [Harvard Med. Sch., Vincent Memorial Hosp., and Massachusetts Gen. Hosp., Boston, Mass.] *New England J. Med.*, 233:11-17. 1945.

A review article in the Medical Progress series, with 48 references.—C. W.

Conization and Early Diagnosis of Carcinoma of the Cervix. HABER, J. J. [Charleston Gen. Hosp., Charleston, W. Va.] *Am. J. Surg.*, 67:68-76. 1945.

Review of the literature and description of the method of conization used at the Charleston General Hospital are given. This is carried out with the electrosurgical unit loop, and the extent of the procedure is classified as slight, moderate, or radical. The procedure is recommended for cervical erosion, for chronic cystic cervicitis, in cases in which amputation of the cervix, Sturmdorff's operation, or trachelorrhaphy is indicated, and before subtotal hysterectomy. Specimens obtained by conization can be used for biopsy purposes. Of 311 patients on whom conization was performed, 18 had carcinoma of the cervix, 11 had squamous metaplasia, and the remainder had other benign lesions. None was adversely affected by the procedure. Four of the carcinomas were disclosed by the conization.—W. A. B.

Cancer of the Cervix Uteri. Some Fundamental Considerations. MARTZLOFF, K. H. [Univ. of Oregon Med. Sch., Portland, Oreg.] *West. J. Surg.*, 53:255-267. 1945.

A review with 97 references. The proper use of radiation and surgery combined, offers a better outlook than either used alone.—M. E. H.

The Diagnosis and Treatment of Carcinoma of the Fundus Uteri. SCHMITZ, H. E., SHEEHAN, J. F., and TOWNE, J. E. [Mercy Hosp., and Loyola Univ. Clin., Chicago, Ill.] *Illinois M. J.*, 87:194-197. 1945.

The vaginal smear method of diagnosing early hidden carcinoma of the uterus holds great promise for the future. X-ray and radium, properly administered, are capable of completely destroying early adenocarcinoma of the uterine fundus.—M. E. H.

MALE GENITAL TRACT

Carcinoma of the Prostate Gland, and Benefits of Diethylstilbestrol or Orchiectomy. WATTENBERG, C. A. [Washington Univ. Sch. of Med., and Barnes Hosp., St. Louis, Mo.] *J. Missouri M. A.*, 42:482-485. 1945.

In the author's plan of treatment diethylstilbestrol is used as soon as the diagnosis of prostatic cancer is made.

Orchiectomy is advised when the patient has signs of metastasis, the serum acid phosphatase is high, the prostatic tumor is enlarging while the patient is taking sufficient doses of diethylstilbestrol, or the patient does not tolerate full dosage of this estrogen. There is no evidence that diethylstilbestrol, or castration, or both can cure carcinoma of the prostate gland.—M. E. H.

Testicular Tumors. VERMOOTEN, V. [Brooke Gen. Hosp., Fort Sam Houston, Tex.] *Arch. Surg.*, 50:63-66. 1945.

Sixty-two testicular tumors were discovered at an Army Hospital in 2 years. Of these, 18% were benign. Radical orchidectomy, followed by roentgen therapy, was performed in 51 patients with malignant tumors, of whom 36 (72%) were alive without clinical evidence of metastasis at the time of writing; 16 of these 36 were followed for 1 to 2 years after operation.—W. A. B.

Carcinoma Derived from Adult Seminiferous Epithelium. A Review of the Literature and a Report of a Case. STOFER, B. E. [Receiving Hosp., and Wayne Univ. Coll. of Med., Detroit, Mich.] *Arch. Path.*, 40:68-71. 1945.

Five photomicrographs show the character of the seminoma in the additional case reported.—J. G. K.

Adrenal Cortical Adenoma of the Epididymis. FREEMAN, A. [St. Luke's Hosp., Chicago, Ill.] *Arch. Path.*, 39:336-337. 1945.

Report of a case.—J. G. K.

SALIVARY GLANDS

Mixed Tumors of the Salivary Glands. HELLWIG, C. A. [St. Francis Hosp., and Sedgwick Co. Tumor Clin., Wichita, Kans.] *Arch. Path.*, 40:1-10. 1945.

A study of 82 primary tumors of the salivary glands is reported together with numerous references from the literature and discussion of the origin and character of the growths.—J. G. K.

GASTROINTESTINAL TRACT

Short Oesophagus (Thoracic Stomach) and Its Association with Peptic Ulceration and Cancer. SMITHERS, D. W. [Roy. Cancer Hosp. (Free), London, England] *Brit. J. Radiol.*, 18:199-209. 1945.

The literature on short esophagus and its relationship to peptic ulceration is reviewed, and the theories regarding this association are discussed. A theory is put forward suggesting that both congenital shortening of the esophagus and acquired shortening due to cicatrization following ulceration are comparatively rare, and that the majority of the cases diagnosed as short esophagus radiologically are primarily cases of hiatus insufficiency associated with spasm of the longitudinal muscle fibers, resulting from irritation due to flow of gastric juice into the esophagus. A lax hiatus may result either from a developmental deficiency due to delayed descent of the stomach or to a loss of elasticity of the tissues in later life. In most cases of "congenital short esophagus," the condition is not congenital and the esophagus is shortened only by spasmodic contraction. Three certain cases and one doubtful one of cancer occurring in a short esophagus are described.—M. L.

Carcinoma of the Esophagus. A Survey of 332 Cases. BOROS, E. [New York City Cancer Hosp., New York, N. Y.] *Gastroenterology*, 5:106-111. 1945.

The overwhelming preponderance of males in the series is in accord with other reports. The tumors were more numerous in the middle and lower thirds and caused dysphagia and pain as the most prominent symptoms. With radiotherapy there was little reduction in the size of the tumor. Gastrostomy afforded some temporary benefit. The mortality of resection was 25%, and there were no cures in this series.—E. E. S.

Carcinoma of the Esophagus. Transpleural Resection and Esophago-Gastrostomy. KROSS, I. [City Hosp., New York, N. Y.] *Am. J. Digest. Dis.*, 12:344-346. 1945.

The article is largely devoted to a detailed description of the operative removal of a squamous carcinoma involving the distal end of the esophagus. Contiguous lymph nodes were invaded. The patient died about 1 year later with many distant metastases.—E. E. S.

Benign Tumors of the Esophagus. Report of Three Cases. ADAMS R., and HOOVER, W. B. [Lahey Clin., Boston, Mass.] *J. Thoracic Surg.*, 14:279-286. 1945.

The authors offer some generalizations based on 97 cases reported in the literature and 3 observed in their own hospital. Dysphagia may be the first indication of the presence of a tumor, but it appears late in the period of growth. Proper roentgenographic studies usually reveal a mass, smooth in contour and movable on swallowing. Warning against biopsy is stressed since infection, swelling, and obstruction may ensue. The mode of extirpation is discussed.—E. E. S.

Survival after Gastric Resection in Carcinoma of the Stomach. CUSTER, W. C. [Highland-Alameda Co. Hosp., Oakland, Calif.] *Surgery*, 17:510-511. 1945.

The data on 96 patients subjected to gastric resection for carcinoma of the stomach in 14 years are summarized. The tumors were all considered operable at the time of laparotomy. The operative and postoperative mortality was 11.39%. Twenty-three patients, or 23.95%, did not survive more than 3 years; these all had extension of the lesion with metastases. Of the entire group, 37.59% died in the 3 to 5 year periods; 27.06% survived the 5 year period; 18.75% survived more than 8 years.—W. A. B.

Emergency Gastrectomy for Acute Perforation of Carcinoma of the Stomach, with Diffuse Soiling of the Free Peritoneal Cavity. BISGARD, J. D., and OVERMILLER, W. [Omaha, Neb.] *Ann. Surg.*, 120:526-530. 1944.

The authors present a case of perforated gastric cancer treated by subtotal gastrectomy, refer to 217 cases from the literature, and discuss the management of the condition. Obtaining a biopsy from the region perforated is advocated because, in many instances, the malignant character of the lesion is not recognized at operation. Resection is not indicated in approximately one-half the cases because of advanced peritonitis or extent of the carcinoma. Only 9 of 43 patients with simple closure of the perforation survived, while 13 of 15 patients with

primary resection recovered. Although successes rather than failures are more likely to be reported and more favorable cases are selected for operation, it is felt that primary gastrectomy in such an emergency is the desired procedure.—W. J. B.

BONE AND BONE MARROW

Osteoid Osteoma. With Case Reports. HAMILTON, J. F. [Willis C. Campbell Clin., Memphis, Tenn.] *Surg., Gynec. & Obst.*, 81:465-474. 1945.

Five cases are presented, and the clinical and pathological aspects of the disease are discussed.—J. G. K.

Dermatomyositis-Like Case of Plasmacytoma with Enormous Hyaline Deposits (Paramyloid). JØRGENSEN, K. S. [Kommune Hosp., Copenhagen, Denmark] *Acta path. et microbiol. Scandinav.*, 21:896-913. 1944.

A case is reported in which the diagnosis was based on the finding of 20% plasma cells in the marrow obtained by sternal puncture, but in which no plasma cells were found at autopsy.—M. H. P.

On the Pathogenesis of Renal Failure Associated with Multiple Myeloma. Electrophoretic and Chemical Analysis of Protein in Urine and Blood Serum. BLACKMAN, S. S., BARKER, W. H., BUELL, M. V., and DAVIS, B. B. [Johns Hopkins Univ., Baltimore, Md.] *J. Clin. Investigation*, 23:163. 1944.

The authors present a detailed report of a patient with multiple myeloma, Bence-Jones proteinuria, and renal insufficiency. In this type of Bright's kidney lesion renal insufficiency depends chiefly on obstruction of tubules by precipitation of Bence-Jones protein. In this patient the serum contained a fraction comprising 24.6% of the total serum protein, which in the Tiselius electrophoresis apparatus had the mobility of a beta globulin. In the urine the protein salted out like a globulin, moved electrophoretically like a beta globulin, and exhibited the solubility characteristics typical of a Bence-Jones protein. While the patient was under observation, the concentration of protein in the urine varied from 0.475 to 0.744 gm. %, and the proportion of Bence-Jones protein usually varied from 92 to 100% of the total. The authors suggest that in multiple myeloma, the development of renal insufficiency caused by the precipitation of plasma proteins within the kidney is determined chiefly by the duration of high concentrations in the urine of proteins that have the solubility and electrophoretic properties of globulins.—J. L. M.

Eosinophilic Granuloma of Bone. MICHAEL, P., and NORCROSS, N. C. [U.S.N.R.] *U. S. Nav. M. Bull.*, 45:661-668. 1945.

The paper gives a brief review of this benign destructive lesion, first described as a clinical entity in 1940, together with reports of 2 cases that occurred in naval personnel. It includes roentgenogram and photomicrograph illustrations. Treatment is excision and x-ray therapy.—C. W.

MUSCLE

Rhabdomyosarcoma. VIETS, H. R., and WITTENBORG, M. H. [Massachusetts Gen. Hosp., and New England Deaconess Hosp., Boston, Mass.] *Arch. Path.*, **40**:179-181. 1945.

Report of a case in which the tumor originated in the muscles of the back, eroded the spinal column, and metastasized to the lungs and kidneys.—J. G. K.

Über das sog. Myoblastenmyom, mit Beschreibung 7 neuer Fälle. [The So-Called Myoblast-Myoma, with Description of 7 New Cases.] RINGERTZ, N. [Caroline Hosp., Stockholm, Sweden] *Acta path. et microbiol. Scand.*, **19**:112-164. 1942.

The clinical picture described by Abrikosoff [*Virchows Arch. j. path. Anat.*, **260**: 215. 1926] as myoma arising from myoblasts is believed by Ringertz to arise from the granular cells of the connective tissue, which infiltrate the muscle. Seven previously unpublished cases of the disease are reported, and the literature is reviewed with an extensive bibliography. Sarcomatous tumors described by various authors as "malignant myoblast-myoma," "myoblastoma," etc., should not be confused with the characteristic Abrikosoff tumors, which are always benign.—M. H. P.

Hemangioma of Tendon. ARKIN, A. M. [Mt. Sinai Hosp., New York, N. Y.] *Am. J. Surg.*, **69**:133-134. 1945.

A case report. The tumor occurred in the tendon of the tibialis anticus, just anterior to the ankle, and had been present for 20 years prior to operative removal occasioned by the onset of pain and swelling.—W. A. B.

PANCREAS

Radical Duodenopancreatectomy. STRODE, J. E. [Honolulu, Hawaii] *Surgery*, **18**:115-129. 1945.

A report of a successful resection of a carcinoma of a duodenal diverticulum involving the head of the pancreas is presented together with a discussion of some aspects of the physiology of the external secretion of the pancreas.—W. A. B.

Resection of the Duodenum and Head of the Pancreas for Primary Carcinoma of the Head of the Pancreas and Ampulla of Vater. COLE, W. H., and REYNOLDS, J. T. [Univ. of Illinois, Coll. of Med., Chicago, Ill.] *Surgery*, **18**:133-143. 1945.

The operative procedures previously described by Whipple, Orr, Brunschweig, and Child are outlined, and a modification of these technics presented. This consists of a one stage resection of duodenum and head of the pancreas, with transplantation of the common duct into the jejunum and the performance of an end-to-end gastrojejunostomy so that the food stream will not pass over the transplanted end of the common duct. In the five cases reported, the pancreatic stump was not implanted into the jejunum, and 2 of the patients developed pancreatic fistulas. There was 1 death in the immediate postoperative period; of those surviving, 2 are well at 4 and 14 months postoperatively.—W. A. B.

Pancreaticoduodenectomy for Carcinoma of the Ampulla and Ampullary Region. ORR, R. G. [Univ. of Kansas, Kansas City, Kan.] *Surgery*, **18**:144-158. 1945.

The 35 cases of pancreaticoduodenectomy recorded in the literature since 1942 are reviewed and 5 new cases

presented. The author stresses the importance of establishing a correct diagnosis of carcinoma, by biopsy of the papilla if necessary, to avoid a needlessly long procedure where conditions other than carcinoma exist, i.e., benign tumor in the ampulla, stone impacted in the ampulla, and chronic pancreatitis. In cases of carcinoma the radical pancreaticoduodenectomy is indicated since this tumor is slow-growing and late to metastasize. Restoration of the external secretion of the pancreas by implantation into the intestinal tract is advocated and a two stage operation is recommended for generally debilitated patients.—W. A. B.

The Origin and Growth of an Adenoma of the Islands of Langerhans. GOOD, L. P. [Texarkana, Tex.] *Surgery*, **18**:159-171. 1945.

The existing hypotheses on the origin of adenomas of the islets are discussed, and the author presents the photomicrographs of one case occurring in a man of 70 years, which support his theory that the origin of the capsule of the tumor is the duct wall.—W. A. B.

A Method of Implanting the Pancreatic Duct into the Jejunum in the Whipple Operation for Carcinoma of the Pancreas. VARCO, R. L. [Univ. of Minnesota Hosp., Minneapolis, Minn.] *Surgery*, **18**:569-573. 1945.

A report of a technic involving the use of a catheter in anastomosis between the pancreatic duct and jejunum is presented. Description of the procedure in one case is given.—W. A. B.

THYROID

Latent Primary Carcinoma of the Thyroid Gland. MITCHELL, N. [Mt. Morris Tuberc. Hosp., Mt. Morris, N. Y.] *Arch. Path.*, **39**:331-335. 1945.

A case report.—J. G. K.

CANCER CONTROL AND PUBLIC HEALTH

Cancer Control in the USSR. *Am. Rev. Soviet Med.*, **3**:191. 1945.

Before the war 3 separate groups were engaged in tumor research. These were the Leningrad school headed by Nikolai Petrov, the Ukraine group directed by Bogomolets, and the Moscow Center in the charge of Peter Herzon.

The program for 1945-1946 includes the establishment of anticancer societies in every large Soviet town. These will be State organizations, and provide free treatment. Compulsory health examinations have been instituted for large groups of the population. It is hoped that this extensive undertaking adopted by the government will make early cancer diagnosis a reality. Consultation centers in 300 different urban areas have been opened, where all cases or suspected cases register. In addition, 150 radiotherapeutic centers have been built. General hospitals all over the country have set aside some 15,000 beds for cancer patients. Free State nursing homes have been established for patients from rural districts. A large number of medical men hope to visit England and the United States to study cancer control and treatment. The development of research is being encouraged by extensive grants to promising research fellows.—J. H.

Book Reviews

CANCER OF THE COLON AND RECTUM. Its Diagnosis and Treatment. Fred W. Rankin, and A. Stephens Graham. First Edition. Springfield, Ill.: Charles C. Thomas. 1945. x + 358 pages. Price \$5.50.

The recent publication of a second printing of Rankin and Graham's *Carcinoma of the Colon and Rectum* attests to the esteem in which it is held by the profession.

It is a work to which the internist may turn for what he must know about diagnosis. The surgeon who only occasionally does this sort of work may lean heavily upon it with confidence and even those whose "series" are large would do well to check their procedure occasionally against General Rankin's advice to prevent the creeping in of bad habits of omission or commission.

Part I on general considerations presents a lucid review of the anatomy of the region and concludes with a valuable section on the neglected subject of physiology. A chapter on incidence occurrence and etiology follows with sobering statistics on the frequency of the disease and the rôle of the polyp in its origin.

Perhaps the most valuable chapters are those on pathology, symptoms and diagnosis. They are presented in classical fashion, are richly illustrated and contain little of a controversial nature.

Such material is to be found, however, in "operability and prognosis" particularly in the basing of the latter so much on microscopic grading especially of biopsy specimens. The former depends upon "surgical judgment" often rather than a set of rules. The authors counsel courage and common sense after consideration of all the data obtainable.

Their choice of operation favors, for the right side a two stage procedure with end to side ileocolostomy by the senior author's closed method, followed by a subsequent resection of the right half of the colon. They admit preference of some for a one stage procedure which, if done, they believe should be complemented by ileostomy proximal to the anastomosis. For the left colon obstructive resection is endorsed without preliminary cecostomy. The methods of others are generously discussed but disapproved. For the rectum they choose the one stage abdomino-perineal or perineo-abdominal operation. They mention the anterior resection but emphasize that it is indicated for the rare favorable case or for the patient who is adamant against the permanent colostomy.

All this unquestionably represents the best opinion of ten years ago but recently it has been announced that the Miller-Abbott tube and the sulfonamides have revolutionized the surgery of the lower bowel. Today, one stage resection and primary anastomosis seems to have won out for either side of the colon while the Miller-Abbott tube provides the safety valve for the right side and sometimes, if there has been no obstruction, for the left side as well. Most surgeons would agree with the text, however, about the abdomino-perineal as the most useful procedure for rectum and recto-sigmoid but admit an increasing interest in the reports of the anterior resection.

Hodges' chapter on radiotherapy reports some astonishing results which he counterbalances with the advice that surgery is preferable. If radiotherapy really has anything to offer the inoperable case it deserves more attention than it now receives.

The chapter on preoperative and postoperative treatment

was carefully written to include every detail of this important phase of the care of patients with large bowel cancer. Since it was written, Dr. Rankin's lack of enthusiasm about intraperitoneal vaccines has been confirmed and the sulfonamides and antibiotics have proven so effective as to rob postoperative peritonitis of much of its threat. Simultaneously, the emphasis on fluid administration has shifted to the colloids. Although the importance of the crystalloids has not been forgotten, today plasma, the casein hydrolysates and blood receive much more attention as the dangers of hypoproteinemia are better appreciated. The Miller-Abbott tube, by solving so much of the decompression problem, is a third development which has radically changed current treatment.

Spinal anesthesia by the method of serial injection has been improved enough to overcome most of the objections held against it a few years ago. The increased number of experienced anesthetists made available by the demobilization of the army and navy restores this valuable method to our consideration. No one can deny that spinal anesthesia makes it easier to do the nicer work demanded of surgeons today.

The last part of the book begins with an interesting historical sketch. It then proceeds to give a step by step description of all the procedures used in large bowel surgery. Each one is evaluated by the authors who supply the evidence that condemns or endorses the operation and who state in what circumstances it should or should not be used.

The post-war surgeon has new weapons with which to attack his old problems. To counsel him as to how these should change his accepted procedures in the field of large bowel cancer, Rankin and Graham are peculiarly qualified by their capacity for sound criticism and clear writing. The second edition of their work will be looked forward to with keenest anticipation.

DAVID C. BULL

CANCER OF THE SCROTUM IN RELATION TO OCCUPATION. S. A. Henry. London: Oxford University Press. 1946. 158.

This monograph is well illustrated with photographs of scrotal cancer and of men engaged in various occupations in which there is a special liability to it. The author has collected an immense amount of statistical and historical information about this form of cancer and is able to draw upon his own experience as an Inspector of Factories. He makes an interesting attempt to assign the most likely etiological factors to the various occupations, and in addition to such well established factors as soot, tar, pitch, and lubricating oils he attaches importance to "heat with radiations" and considers this agent to be of importance not only in such occupations as makers of glass, furnacemen and rollers, smiths and skilled forgemmen, but also to such occupations as that of grocer and butcher, where the liability to excessive heat with radiations is not very obvious. The later sections of the book, dealing with the time necessary for the production of the primary growth, contain by far the most elaborate study that has ever been made of the range of the incubation period of induced cancer in man.

E. L. KENNAWAY